[redacted] SECURITY INFORMATION

[redacted]

WAR DEPARTMENT

CHEMICAL WARFARE SERVICE

RESEARCH DIVISION

AMERICAN UNIVERSITY EXPERIMENT STATION

WASHINGTON, D. C.

MAJOR GENERAL W.L. SIBERT, DIRECTOR

COLONEL G.A. BURRELL, CHIEF OF RESEARCH DIVISION

CLEARED
For Public Release

CHEMICAL WARFARE MONOGRAPHS

VOLUME THIRTY SEVEN

RICIN

[redacted] REID HUNT

[redacted]
Downgraded
Authority of
CO USA Bio Labs, Ft
Detrick, Md 24 Jul 64

JOSEPH M. CLUTCHKER, Chairman
Classified Document Review Committee
CRDL, Edgewood Arsenal, Md.
Date 5 Aug 64

[redacted] CANCELLED
SECURITY INFORMATION

[redacted]

[redacted] DOD DIR 5200.10

TECHNICAL LIBRARY
ARMY CHEMICAL CENTER

RICIN.

Reid Hunt

(b) (6)

September 30, 1918.

TABLE OF CONTENTS

P R E F A C E

The following report on Ricin was prepared by Dr. Reid Hunt. It includes a summary of the results obtained by him and his co-workers (b) (6) together with those obtained by (b) (6) working at the American University. Reference is also made to previous work on Ricin, which may be of interest in the present connection. Negative results are also reported, briefly, in the hope that they may make it easier for subsequent investigators to select the most promising lines of work.

(b) (6)

INTRODUCTION.

It has long been known that castor beans contain a very active poison which does not pass into the oil. The poison seems to be present in all parts of the seed except the hull (See Werner, Kobert, Cornevin, v. Eisler and Portheim, Durham, Cruz, Agulhon). After many years of discussion (papers by Dixson, Stillmark, Cushny, F. Muller, Jacoby, Rochat, Brieger, Kobert, Oenheimer, Osborne and associates, Woronzow) it has been concluded, largely as the result of the work of Cushny and of Osborne and his associates, that the poison is inseparably connected with a protein and is most probably itself an albumin. The theory that ricin is a ferment has been abandoned (Brunner, Cushny, Liebermann). All recent methods of extracting and handling the poison are based on its behavior as an albumin; earlier methods were based upon the view that the poison was either a globulin or was closely associated with a globulin. (Methods of Kobert and Jacoby).

In addition to the specific poisonous albumin, ("ricin") the castor seed contains much larger amounts of a non-toxic globulin (Ritthausen, Osborne), and non-toxic proteoses (Cushny, Osborne) among its nitrogen containing substances.

It has not been possible to isolate ricin in a condition of purity. There are, also, no chemical tests for its identity.

The only criterion for its presence and approximate amount are its effects upon biological structures. These effects manifest themselves in various ways:(1) the toxicity, i.e., the ability to kill animals or to cause in them certain definite pathological changes, (2) its effects upon red blood corpuscles, (3) certain immunological reactions due to the fact that animals can be immunized against ricin just as they can be immunized against certain bacterial toxins. All attempts at quantitative determinations of ricin are based upon one or the other of these physiological reactions. Up to the present time the toxicity method has been almost exclusively employed. There are, however, a number of objections to this method, as will appar in subsequent parts of this report. Among the most serious of these the following may be mentioned here: the method is very time consuming, many animals are required, and results can only be obtained very slowly. As it was necessary to check every step taken in the extraction of the crude material and the isolation of an active preparation, as well as to determine the keeping properties of the latter and the effects upon it of various manipulations, our first experiments were directed to determining whether the second of the above biological effects (the effect upon red blood corpuscles) could be utilized as a quantitative measure of the toxicity of a given preparation.

QUANTITATIVE TESTS FOR RICIN BASED UPON THE AGGLUTINATION OF RED BLOOD CORPUSCLES:

When a solution of a preparation containing a small amount of ricin is added to diluted defibrinated blood or to a suspension of red blood corpuscles in an isotonic sodium chloride solution (or certain other solutions; see Field; Girard-Mangin and Henri), the red blood corpuscles agglutinate, or clump together, and settle to the bottom of the container leaving a clear, colorless, supernatent liquid. The red blood corpuscles in defibrinated blood readily pass through filter paper; the agglutinated ones do not (Stillmark). The agglutination may also be observed directly under the microscope (Cruz and others).

The remarkable property of ricin was discovered by Stillmark (1889) and has been the subject of a very large number of researches (on the nature of the reaction see Stillmark, Kobert, Kraus, Landsteiner, Liebermann, etc.). Methods of detecting ricin (or castor beans) in cases of suspected poisoning, especially by feeding materials, have been based upon this agglutinating action (Kobert, Fuhner, Miessner and Rewald). It has, however, been found (Guyot, Landsteiner and associates, Raubitschek and associates, Pendel, v. Eisler and Portheim) that extracts of various non-toxic seeds, as well as a number of poisons analogous to ricin (abrin, crotin, robin), have a similar agglutinating action on red blood corpuscles although acting very unequally on different kinds of blood; more trustworthy metho

for detecting ricin are based upon the toxicity and certain immunity reactions to which reference will be made later. However, if it is simply a question of determining the presence or absence of ricin, the agglutination test is valuable. Kobert recommends the following procedure which may be useful as a simple, largely qualitative test: the ground seeds are extracted with 0.9 precent sodium chloride solution (we would recommend 2 cc of the solution to 1 gm. of the meal) alcohol is added to complete precipitation; the precipitate is rapidly filtered and dissolved in saline solution, 10 cc portions of various dilutions are added to a mixture of 9.8 cc 0.9 percent sodium chloride and 0.2 cc defibrinated blood (rabbit, dog, guinea pig, man, cat, rate, etc.).

It was thought for some time that the toxic action of ricin was due to this action upon the red blood cells, but this view has been disapproved (Flexner, Cushny, Cruz, F. Muller), and it is now recognized that the poison directly affects organs and tissues in a way very similar to that of certain bacterial toxines. Ricin also agglutinates various other cells (liver, brain, epithelial etc.) when they are suitably separated (Lau, Kobert, Michaelis and Steindorff, Reid, Levaditi and Mutermilch).

There has been a great deal of discussion as to whether there are two active substances in "ricin", an "agglutinin" and a "toxin" or whether the agglutinating and toxic actions are due to the same substance. Among

the arguments which may be advanced for the presence of but one active substance the following may be cited: the seeds of a large number of varieties of the plant, from all parts of the world, have been examined (Stillmark, Assmann, Agulhon) and all found to contain both toxic and agglutinating properties and in the same relative amounts; with few exceptions (noted below), preparations with high agglutinating activity also had a highly toxic action (Osborne, Mendel and Harris and others) and vice versa; various adsorption products are both toxic and agglutinating (Landsteiner and Raubutschek); the two properties are destroyed pari passu by heat (Miessner and Miessner and Rewald) and other physical agents (ultra - violet light: Bareni and Jonesco-Mihaiesti; flourescent substances in the presence of oxygen: Tappeiner, Jodlbauer); immune sera, prepared by injecting ricin into animals, always inhibit both the toxic and agglutinating (and other) actions of ricin (Ehrlich, Danyzs, Jacoby), although Madsen and Walbum found support for the view that there are present two substances from a detailed analysis of experiments with antiricin. On the other hand, it has been claimed that by digestion with pepsin the agglutinating property was more easily destroyed than was the toxic property (F.Muller, Rochat, Jacoby, Michaelis and Oppenheimer); that during the germination of the seed the agglutinating

property disappeared first (Agulhon); that on prolonged preservation the agglutinin may remain but the toxin disappears (Field; see, however, Levaditi and Mutermilch and our own experiments); that under the influence of the electric current a preparation especially rich in agglutinin may be obtained (Field; cf. Landsteiner and Pauli); that the agglutinin may be absorbed by red blood corpuscles and so removed from the solution which continues to be toxic (Liebermann, see, however, Rehns, Reid). Many of these arguments are open to criticism (see e.g. Lau, Osborne, Mendel and Harris, Brieger, Reid, Miessner). In any case, the evidence at present available indicates that there is, except perhaps under distinctly artificial conditions, a parallelism between the agglutinating and toxic properties of ricin and our first experiments were directed to determining whether a quantitative method for determining the toxicity of a preparation could be based upon the preparation's activity in agglutinating red blood corpuscles. (There has been considerable discussion as to whether "ricin" has also a lipolytic action: Neuberg, Neuberg and Rosenberg, Mendel and others; the evidence is that a pure preparation does not have such an action).

Method:

(a) Choice of Blood: The red blood corpuscles of all vertebrate animals tested are agglutinated by ricin; the statements that the corpuscles of the goat and of a barbel are not agglutinated have been proved incorrect (Jacoby, Miessner and Rewald; Fraenkel). Various authors have reported wide variations in the ease with which the corpuscles of various animals are agglutinated(Stillmark, Elfstrand, Lau, Jacoby, Landsteiner, Raubitschek, v. Eisler, Field, Rehns, Assmann, Miessner and Rewald). Although marked variations probably exist, the figures published have comparatively little value, owing to the differences in the technique employed. Thus, women workers used hypertonic salt solutions which alone may agglutinate, or contribute to the agglutination of, certain corpuscles; some have used "washed" corpuscles, others, corpuscles suspended in blood serum. In most cases blood serum has an inhibitory action upon ricin agglutination (Stillmark, Kobert, Fraenkel, Miessner and Rewald, Raubitschek), but the serum of different animals varies greatly in this respect and that of the beef is said to have the opposite effect and to accelerate the ricin agglutination (Miessner and Rewald). Nevertheless the results seem to show that the corpuscles of the horse and sheep (v. Eisler) and especially the goat are less easily agglutinated than are those of other mammals

tested; Landsteiner tabulated the ease with which certain blood corpuscles are agglutinated as follows (beef most resistant):

Beef Blood	1
Sheep "	1
Horse "	2
Fowl "	4
Hog "	8
Pigeon "	8

Elfstrand gave the following order:

Sheep
Hen
Dog
Man
Cat
Rabbit
Swine
Pigeon
Guinea Pig

Miessner and Rewald obtained very nearly the same order. In view of the contradictory results published, it is desirable that the kind of blood, the number of corpuscles per cubic millimeter (see below), as well as the method of determining the end point, should always be stated in reporting results.

Employing the technique described below, based upon the number of corpuscles present, we obtained with a ricin preparation the following results:

TABLE I.

Dilution of ricin for

Corpuscles of	Complete Agglutination	Incomplete Agglutination
Sheep	1:12000	1:15000
Dog	1:18000	1:20000
Human	1:20000	1:28000
Guinea Pig	1:20000	1:28000
Rat	1:28000	1:40000

Rabbit corpuscles have also given entirely satisfactory results (Madsen and Walbum and many others). All of our routine experiments have been made with dog's blood and this blood is recommended because of the ease with which satisfactory amounts may be obtained without injury to the animal.

(b) Concentration of red cells: We found no record in the literature of strictly quantitative studies of the action of ricin upon the blood corpuscles. That a very small amount of ricin will agglutinate a very large number of corpuscles is evident; thus Cruz had a preparation of ricin 0.125 mg. of which agglutinated 5 cc. of defibrinated blood (probably about 25,000,000,000 corpuscles). Girard-Mangin and Henri record agglutination in 5 cc. of a 10 per cent blood corpuscle suspension from one drop of a 1 per cent soultion of a crude ricin preparation. That there is a relation between the number of corpuscles agglutinated and the amount of ricin employed is indicated by such

experiments as those of F. Muller who found that 0.01 cc. of a 1 per cent solution of a commerical preparation of ricin agglutinated an equal amount of a 5 per cent suspension of rabbits' corpuscles, whereas smaller amounts had less or no effect. (See also Liebermann and Landsteiner and Reich).

In our work we have taken as a basis the amount of ricin which completely agglutinated, within a given time, 2 cc. of blood containing 200,000 corpuscles per cubic millimeter; the method being to dilute the blood so that it would contain 400,000 corpuscles per cubic millimeter and then adding 1 cc. of the ricin solution to 1 cc. of the blood dilution. Working in this way we had an experiment, for example, which indicated that if 1 cc. of a 1:1000 solution of a given preparation of ricin would agglutinate 1 cc. of blood containing 400,000 red cells per cubic millimeter, 0.1 cc. would agglutinate 1 cc. of blood containing 40,000 red cells per cubic millimeter.

(c) <u>The Suspension of the Red Corpuscles</u>: The suspension of red corpuscles for agglutination tests may be prepared in various ways. Some have used defibrinated blood suitably diluted with isotonic sodium chloride solution; others (Ehrlich) have used blood prevented from clotting by the addition of sodium citrate (0.5 Gm sodium citrate / 95 cc. physiological saline solution / 5 cc. blood drawn directly from a blood vessel). Since the serum of most animals inhibits to a greater or less extent the agglutinat-

ing action of ricin (see above), many workers have used corpuscles which have been "washed" in the centrifuge with an isotonic solution of sodium chloride and then suspended in the latter; the reaction is as a rule rendered more sensitive by this procedure. For the comparisons in which we were interested, diluted defibrinated blood proved ~~ob~~ entirely satisfactory; it was prepared as follows: Ten to twenty cc. of blood (taken from a dog by cardiac puncture) was defibrinated by shaking with glass beads. The blood was filtered and after shaking, the red corpuscles were counted; the blood was then diluted with 0.9 percent sodium chloride solution so that 1 cubic mm. contained about 400,000 cells. (Sodium chloride, not Locke's solution should be used in making the dilution; there was a greater tendency for hemolysis to occur when Locke's solution was used. In this connection, attention may be called to the fact that under certain conditions not fully understood, the agglutination of the red cells may be accompanied or followed, by more or less hemolysis: Ehrlich, Baumgarten, Fraenkel, Pascucci, Mendel, Olmer and Sauvan, Liebermann, v. Eisler and Portheim, Girard-Mangin and Henri, Sachs; With our technique and the quantities of ricin used, hemolysis did not occur. There is also considerable literature on the effects of various chemicals upon the process of agglutination: Ehrlich, Jacoby, v. Eisler and Tsuru, Girard-Mangin, Henri, Kudo, Jodlbauer,

Rehns, etc; the results seem to have no special interest in the present connection except perhaps those of Liebermann, according to which formaldehyde delays, but does not prevent, the agglutination).

(d) End Point: An attempt was made to determine an agglutination end point by use of the microscope. This was found to be extremely unsatisfactory, however, as no two similar results could be obtained. Much more dependable results were secured by passing the mixed diluted blood and solution of ricin through filter paper. The totally agglutinated cells do not pass the filter paper readily and thus a clear filtrate, usually without traces of color, results. Partly agglutinated cells give a filtrate of varying degrees of cloudiness with greater and greater depth of color from the cells which are not in sufficiently large aggregates to be held back by the paper. It was much simpler to determine, by this method, total agglutination than any degree of partial agglutination; a result was not recorded as "total agglutination" unless the filtrate was absolutely free from any visible cloud.

(1) Filter Paper: Much depends on the filter paper in determining the above end point. Our first quantitative work was done with a German filter paper of which only a limited supply was available; later "Whatman No. 5" was secured. It was found that the latter paper was much more effective in holding back the smaller

agglutinated masses and thus gave clear filtrates in much more dilute solutions of ricin. Variations in filter paper, therefore, greatly interfere with accurate quantitative results and new lots should always be tested out against that which has been used. Even then the individual filters in the same packet also show variations, but no remedy for this defect in the method, which in other respects seems to be the best, can be suggested.

(2) Time in Relation to the End Point: There is some relation, not yet fully investigated, between the time of exposure and the amount of ricin present; a prolonged exposure to a small amount of ricin may give as complete agglutination as a larger amount for a shorter time(Assmann, Agulhan), but much depends upon the kind of blood used (Miessner and Rewald). The reaction with dog's blood is rapid; we found little change between 5 and 80 minutes and during this time the curve expressing the relation between the amount of ricin present and its agglutinating power was nearly a straight line.

(3) Temperature: Variations in the temperature of the mixture of red cells and ricin appears to be without effect on the completeness of the reaction. This has been tested at temperatures varying from 22°C to 38°C and is in agreement with Osborne, Mendel and Harris, who state that incubation at 37° did not materially influence the reaction. Since ordinary changes in room temperature do not introduce a variable, such temperatures may be used for quantitative determinations. (Attention

may be called to the fact that in working with mixtures of ricin, antiricin, and blood it is desirable to use a temperature of 37°; at this temperature an end point may be reached in 10-15 minutes which is not reached for several hours at 15°: Madsen and Walbum).

(e) Technique for Carrying out the Test:

(1) Weigh out a convenient amount of the ricin preparation (20 milligrams) and make a 1% solution in 0.9% sodium chloride solution.

(2) From this 1:100 solution make the following dilutions: 1:500; 1:1000; 1:2000; 1:4000; 1:6000.

(3) Count the corpuscles of the defibrinated blood and dilute with 0.9% sodium chloride solution so that the suspension contains 400,000 red cells per cubic millimeter.

(4) Measure out 1 cc. amounts of the above dilutions of the ricin preparation into 5 perfectly clean test tubes: introduce 1 cc. of 0.9% saline into a sixth tube (control).

(5) Add 1 cc. of the diluted blood to each of the tubes containing 1 cc. of the ricin solution and also to the control tube.

(6) Place filter papers in 6 clean funnels and moisten with 0.9% saline.

(7) At the end of 60 minutes from the mixing of the red cells with ricin, pour the mixture from each

dilution on the filters and observe whether the filtrate collected in test tubes is free from cloud. All the more concentrated ricin solutions should give a perfectly clear filtrate; the weaker ones should show a slight but increasing cloudiness with more and more evidence of red color in the filtrate.

(8) Repeat for checking purposes and average results. Dilutions within more narrow limits may be used if desired.

(f) Application of the above Tests to Different Preparations of Ricin:

We have made three preparations of ricin which may, on account of their availability, prove of special interest: (a) a simple water extract, (b) an ammonium sulphate precipitate, and (c) a picrate. The above method is applicable to all three of these, although the picrate does not lend itself easily to agglutination tests because of its relative insolubility in 0.9% sodium chloride solution.

The advantage of this method as a quantitative test for ricin is well shown in some of our work in attempting to secure very active ammonium sulphate precipitates by pressing such precipitates nearly to dryness, thus removing a considerable amount of ammonium sulphate dissolved in the expressed water. A test was made and

completed within two hours, whereas toxicity tests were incomplete after 4 days.

(g) Parallelism of Agglutination and Toxicity Tests:

That, in general, a preparation of ricin with a high agglutinating activity has also a high toxic value is shown by the following figures. The preparations were made by widely different methods; some in Boston, some in Washington, and from different meals.

TABLE II.

Specimen	Amount to agglutinate 1 cc of standard blood suspension	Fatal dose per K white mouse; death within 24 hours
G-W	0.05 mgm.	0.35
F-B	0.25 "	1.66
		death in 48 hours
B-B	0.24 "	0.71
F-B	0.25 "	0.64
G-B	0.25 "	0.62
E-B	2.5 "	2.75

With doses which did not cause death so acutely the parallelism was not always so close; perhaps in these cases secondary factors, varying with different animals, were involved. Field found that individual variations in the susceptibility of the animals became an extremely disturbing factor when doses were given which did not kill within six days.

TABLE III.

Specimen	Amount to agglutinate 1 cc blood suspension	Fatal dose in 72 hours
G(W)	0.05	0.12
C(W)	0.12	0.275
B(B)	0.24	0.25
H(B)	0.25	0.45
J(B)	2.0	1.32
E(B)	2.5	0.74
F_2(B)	5.0	3.85

Our experience has been that the agglutinating activity of a preparation is a very useful guide for the determination of the efficiency of extraction, as well as also giving a very reliable indication of the toxicity of the preparation. In fact, there are indications that with a sufficient number of experiments to eliminate individual variations, a factor based upon time of death would be obtained which would be a constant.

THE EXTRACTION OF ACTIVE PREPARATIONS OF RICIN:

The following experiments relate largely to the extraction of commercial bean meal; the influence of the residual fat content, the fineness of the powder and other factors will be discussed later.

General Principles:

The proteins of the castor bean consist of two or more globulins, two or more proteoses and an albumin which is either ricin itself or from which the ricin has not as yet been separated. The albumin constitutes but a small part (less than 1 percent) of the

total weight of the seed. The solubility of ricin in water, or very dilute salt solutions, and the insolubility of the globulins in these solvents are the basis for the preparation of active specimens of ricin. Before taking up our own work, brief reference may be made to the methods most successfully employed in the preparation of active specimens of ricin and upon which the conclusions as to the nature of ricin are based (Osborne and associates): the ground beans, fat-extracted, were extracted with 10% sodium chloride; by this means not only the ricin but all other soluble proteins were extracted. The solution was dialyzed for four or five days, by which process much of the globulin was precipitated, whereas the ricin remained in solution and some of the proteose passed out in the dialysate. The solution was filtered and to the filtrate was added enough ammonium sulphate to bring it to a 45% concentration; by this means the ricin and the remaining globulin were largely precipitated, whereas much of the proteose remained in solution, although a certain percentage also was precipitated. The precipitate, after filtration, was dissolved in water and an equal quantity of saturated ammonium sulphate solution added. The precipitate was removed by filtration, dissolved in water and enough saturated ammonium sulphate added to make a 33 percent satu-

rated solution by which the ricin and remaining globulin were again precipitated. The precipitate was dissolved in water and dialyzed for seven days against running tap water and then against distilled water for seven days. The solution was filtered from the precipitated globulin and evaporated to dryness in vacuo. By this procedure Field obtained a preparation consisting of 75.46% coagulable albumin ("ricin") and 24.54% of non-toxic proteose. The fatal dosesof this preparation per Kilo by intramuscular injection were as follows (time of death not stated in most cases):

TABLE IV.

Rabbit	0.0001 mgm.	
Cat	0.0002 "	
Dog	0.0006 "	
Guinea Pig	0.0008 "	
Goat	0.003 "	(death in 3 days)

Thus, an extraordinarily toxic preparation may be obtained by the above method, but the method is obviously not suited for large scale production.

Our efforts were directed to finding a method by which preparations of a toxicity sufficient for the purpose in view could be obtained and which would be feasible on a large scale. The experiments were made along two main lines: (1) a simplification of the above method of preparation and (2) attempts to absorb the ricin. It was necessary, in either case,

to prepare an extract of the meal and our first experiments were directed to finding the best method of obtaining such an extract.

THE SOLVENT:

The most active preparation of ricin has been made from extracts prepared by treating the meal with 10 per cent sodium chloride solution. Such a solvent, however, extracts a large amount of globulin which must be subsequently removed by the tedious process of dialysis. Since ricin is readily soluble in water, the question at once arose whether there were advantages in using the sodium chloride method. The only data found in the literature on this subject were the statements of Woronzow that the salt solution extracts more of the toxic material than does water. Osborne suggested in explanation of this that the salt solution may break up an insoluble compound of the ricin; that some such factor is involved is indicated by the results of Williams, who found that a quantity of the original meal, when injected subcutaneously, is much less toxic than an extract made from the same amount of meal.

Williams compared the efficiency of water and salt solution extraction and came to the following conclusion:

Salt solution appears to extract somewhat

more of the toxin than does water, but either solvent extracts the toxin fairly efficiently, but the relative concentration of toxin is greater in water extracts, owing to the smaller amounts of globulin removed by this solvent. Our own experience has been that water is a satisfactory solvent and is especially to be preferred for large scale production. We found that Boston city water and distilled water gave identical results; a water supply comtaining a larger amount of dissolved salts might prove inferior to distilled water.

METHOD OF EXTRACTION:

(A) Percolation: It seemed possible that for large scale production a method of percolation, analogous to that used in manufacturing pharmacy, could be utilized. It was thought, further, that a more complete extraction might be obtained by a system of re-percolation. Accordingly, a battery of 3 percolators was set up. It was found that the menstruum did not pass through the meal, no matter how loosely packed, with sufficient rapidity. This difficulty was overcome by adding sand to meal. Very much more concentrated extracts were thus obtained. The upper percolator was packed with a mixture of 1 part meal and 2 parts of sea sand, by weight; the middle percolator with 1 part meal and 1½ parts sand; the lower, with equal parts of meal and sand. After 24 hours percolation tests by agglutination indicated that

the meal of the upper percolator was nearly exhausted since a sample of the percolate collected at this time did not agglutinate in a dilution of 2 to 1 of diluted blood. The meal in the middle percolator was about one half exhausted, a sample of the percolate agglutinating at 1 to 100 of diluted blood. The lower percolate, collected during the 24 hour period agglutinated at 1:400 while the "first running" agglutinated at 1:1400. There are two undersirable results from percolation, however; there is an increase in the amount of globulin due to the increased salt concentration in these more concentrated preparations; the slow exhaustion of the meal favors bacterial decomposition which takes place very rapidly, owing to the albuminous nature of the extraction.

(B) Maceration: Percolation not proving entirely satisfactory, simple maceration with subsequent filtration was tried. A number of factors had to be considered; these will be discussed under the following headings:

a. Relative amounts of solvent and bean meal.

b. Time of maceration.

c. Serial maceration.

a. Relative Amounts of Solvent and Bean Meal: Williams found that a comparatively small volume of water (250 cc to 100 grams meal) left relatively little toxin in the residue which was extracted by a larger volume

(10 liters per 100 grams). This was quite in accord with our experience, although generally 2 parts of water to 1 of meal were found equally satisfactory. Special types of meal required variations from the above, but 2 to 1 macerations are recommended for routine work.

b. The Time of Maceration: necessary to most effectively exhaust the meal was studied by Williams who reported that macerations from the eighth to the twenty-fourth hour gave slightly lower results, judged by the weight of dried extractive than did shorter or longer periods. Our experience does not offer any indications that such a relation is more than incidental since our tests show a very small increase in total solids extracted during the same period. The agglutinating factor also indicates that macerations from 15 minutes to 48 hours exhaust the meal equally well. This is shown by the following table made up from the averages of two tests:

TABLE V.

Time of maceration	Average solids in percent	Average total agglutination
15 Min.	4.61	1.175
30 "	4.88	1.175
1 Hr.	4.83	1.200
2 "	5.07	1.175
4 "	5.16	1.175
8 "	5.25	1.200
12 "	5.06	1.200
16 "	5.56	1.200
24 "	5.03	1.250
36 "	4.69	1.150
48 "	4.40	1.300 *

* In one of these experiments agglutination was at 1:400, which seems to be an error, and this makes the average agglutination probably higher than it actually should be.

c. Serial Maceration: Maceration in series, using the same solvent on several lots of meal, was found to give an extract containing a generally proportional increase in the amount of extractive. The agglutinating power of such extracts does not quite maintain the same increase, due to a constantly increasing amount of globulin. This latter probably becomes increasingly soluble as the salt content of the extracted meal passes into the solvent, thus gradually producing an effect similar to a sodium chloride solution which has so frequently been used to extract the total soluble protein content of the meal. The results of two such serial macerations are shown in the following table:

TABLE VI.

Series I.

Times solvent used on fresh meal	Percent solids	Total agglutination	Agglutination ratio
1	4.40	1:200	100
2	8.40	1:300	150
3	11.95	1:400	200

Series II.

1	5.34	1:200	100
2	10.00	1:400	200
3	16.30	1:500	250
4	22.30	1:600	300
5	29.00	1:800	400

The toxicity of the filtrates from Nos. 1 and 5 of Series II was tested (subcutaneous injection into mice), the fatal doses being given on the basis of solid extractive:

TABLE VII.

	Dose in terms of solid extract.	Time of death
No. 1	0.68 mgm. per Kilo. mouse	2 days
"	1.40 " " " "	2 "
No. 5	1.70 " " " "	2 "
" 1	0.34 " " " "	3 "
" 5	0.87 " " " "	3 "
" 5	0.43 " " " "	5 "

Assuming that this decrease in toxicity is due to the increasing amounts of globulin, serial maceration loses partly some of its advantages, for there is apparently no easy method by which the globulin can be removed.

FACTORS INFLUENCING THE COMPLETENESS OF EXTRACTION OF CASTOR BEAN MEAL:

a. The commercial press cake, after the extraction of the meal in the production of the so-called cold drawn oil, leaves a residue representing about 50 percent of the original meal. This consists largely of cellulose, protein, and residual oil, about 12 percent of the latter remaining in the press cake. The press cake is a somewhat pulverulent mass. The dark green-gray color is due in considerable part to the horny shell-like coating of the seed.

For part of our work, the press cake was further ground to secure a more finely divided powder. After a single passage through a small laboratory drug mill, the meal may be divided into various fractions by sifting. In this way, the hulls are largely removed by the coarser meshed sieves and amount to 40 to 50 percent; they do not contain more than a small percentage of the total ricin, if, in fact, the ricin present is not due to the incomplete separation of kernel and hull. The inactive, or relatively inactive, part amounts to about 50 percent of the press cake as is indicated by the following: After a single passage through a laboratory drug mill, the meal was separated into fractions by sifting: 14% did not pass the No. 20 sieve; 47% did not pass the No. 40 sieve; 14% did not pass the No. 60 sieve; 22% did not pass the No. 80 sieve, and only 3% was finer than No.80.

Since the meals passing the No. 60 sieve formed a pasty mass on the addition of 2 parts of water, 3 parts of water were added to each of the first four fractions with the following results:

TABLE VIII.

Meal	Percent solid	Total agglutination
Over 20 mesh	1.74	1:20
" 40 "	1.55	1:20
" 60 "	4.79	1:200
" 80 "	4.82	1:400

To further test the coarser particles, a sample of over 40 mesh meal was reground and resifted. This gave one lot over 40 mesh, one over 60 and one over 80 mesh sieve. When extracted with 3 parts of water, the over 60 mesh meals both gave incomplete agglutination at 1:20; the fraction over 80 mesh sieve agglutinated at 1:40. In spite of the inactivity of the hull, however, their removal necessitates the use of larger amounts of solvent, so that it is not clear that a gain is made by such a procedure.

The press cake as it comes from the mill affords as active preparations of ricin as does the meal that is ground as described above. An experiment in triplicate gave filtrates without variation in the agglutinating index, although the dilutions would show 10 percent variation. It thus seems that the coarsely ground meal, as it is received from the oil mill, may be

used as advantageously as more finely ground meal.

b. Does the residual fat interfere with the completeness of extraction? A number of investigators have taken care to remove the residual fat from the press cake. Williams concluded as a result of a special experiment that a more potent extract could be secured after removing the oil; however, he compared meal containing as much as 27 percent oil with the same meal after practically all oil had been extracted with ether. Commercial cold press cake is said to contain only about 15 to 18 percent of oil. Williams states that complete removal of the oil is hardly necessary for fairly satisfactory extraction. Our results completely support this latter observation for we were not able to detect any difference in the amount of ricin, determined by agglutination tests, between the aqueous extracts of the natural and of the defatted press cake.

METHODS OF PURIFYING RICIN.

Having obtained, by the method outlined above, an aqueous solution of ricin the next step was to obtain the latter in a more concentrated form. Efforts to do this were carried out along 3 lines: (1) methods of adsorption, (2) methods of precipitation. (A sharp distinction between these two processes can not be drawn). (3) Simple evaporation of the aqueous extract.

(1) Adsorption Methods: Stillmark and Cushny found ricin to be carried down from solutions by a variety of precipitates; Cushny attempted to make use of this property to purify it by means of organic and inorganic precipitates but without marked success. Cushny, Landsteiner and associates, and others have found that ricin is adsorbed by such substances as casein, fibrin, coagulated serum-albumin, silk etc. Tichomiroff found that ricin is carried down completely in the precipitate formed by adding a solution of it containing acetic acid to a solution of nucleic acid. (For other relations of ricin to nuceleic acid see Stassano).

Williams experimented with certain adsorbents which have been successfully used for purifying enzymes. He prepared an aqueous extract of 1200 grams of meal, from which most of the fat had been removed, with 3000 cc of tap water; after filtration the liquid measured 2750 cc. This was divided into 6 portions of about 460 cc each numbered A_1, to A_6. From A_1, an active preparation of ricin was made, according to Osborne's method, by precipitation with ammonium sulphate and dialysis. A_2 was precipitated by mixing with 20 cc of alumina cream containing approximately 11.6 gm. of $Al(OH)_3$ per 100 cc; A_3 was precipitated by 50 cc and A_4 by 10 cc of alumina cream. A_5 was treated with 10 gm. of English fuller's earth and A_6 with 10 gm. of animal charcoal. In

each case, the precipitate was filtered off and dried; ammonium sulphate was added to the filtrate and the precipitate treated as in A_1 . The alumina cream precipitates were inactive, whereas the filtrates from these were very active, i.e., alumina cream does not adsorb the ricin. An animal charcoal and fuller's earth removed some of the ricin, but Williams concluded that the method would probably prove of little value.

We extracted 20 grams of fat free meal with 200 cc 10% sodium chloride solution and added 20 grams of fuller's earth. Nearly all of the ricin remained in the filtrate.

Among the other adsorption methods which we tried, the following may be mentioned:

(A) Insoluble soaps: One cc of a saturated neutral solution of a potassium soap was added to 90 cc. of the filtered macerate from 50 grams of meal. A slight precipitate formed, colloidal in nature, which was not held back by the filter paper. Barium nitrate, strontium nitrate, and calcium chloride were each added to portions of this mixture and in each case a heavy precipitate was formed. These precipitates filtered very imperfectly and, when dried, formed mucilagenous-like masses which adhered to the filter paper and from which they could not be separated. The filtrates were then further clarified by centrifulagation and the supernatent liquids

were tested for agglutination. The results indicated that about half of the original ricin still remained in solution.

(B) Carbonate Precipitates:(cf. Cushny)

a. One gram barium chloride was added to 100 cc of the filtered macerate. A very slight turbidity resulted but, on the addition of 5 cc. of a 10 percent solution of sodium carbonate, a very abundant precipitate formed. This was removed by filtration and the filtrate, which was still turbid, was centrifuged without entirely clearing up the turbidity. The supernatent fluid was tested for agglutination and it was found to represent nearly the activity of the original macerate.

b. Calcium chloride was added to a 2:1 bean meal filtered macerate. Sodium carbonate was then added to precipitate calcium carbonate. The filtrate retained its full agglutinating activity.

c. Strontium nitrate was added to a 2:1 bean meal filtered macerate. Sodium carbonate was then added precipitating strontium carbonate. The filtrate was practically of full activity.

It is clear from the above that the precipitated carbonates do not carry ricin down.

(C) Copper Ferricyanide: Potassium ferri-

cyanide added to the filtered macerate caused no precipitation. Copper sulphate solution was then added, precipitating copper-ferricyanide. The filtrate retained about half its agglutinating activity.

2. Precipitation Experiments:

(A) The Ammonium Sulphate Precipitate:

In almost all the work hitherto done on the isolation of ricin, the starting point was a sodium chloride extract of the bean meal, for it is only since the work of Osborne that it has been recognized that ricin is an albumin or, at least, reacts as if it were an albumin. The sodium chloride renders the globulin, which is present in the beans in large amounts, soluble ; hence the sodium chloride extracts are very rich in globulin, which is not toxic, and an elaborate method of purification is necessary in order to obtain preparations of high activity.

("Mercks Ricin" which has been so extensively used in experimental work is, according to Jacoby, simply the ammonium sulphate precipitate of an 11 percent sodium chloride solution extract of the bean meal; it may contain 30 percent of ash. According to Miessner it has only about the toxicity of the meal itself. A better preparation can be prepared very simply by the process described below).

The demonstration by Osborne of the

albumin nature of ricin suggests a much simpler method of procedure. The albumin is soluble in water; hence, as stated above, water may be used as the extracting agent. Such an aqueous extract should contain relatively little globulin as compared with the sodium chloride extract. Jacoby had shown that ricin is precipitated from its sodium chloride extract by 6/10 saturation with ammonium sulphate; Osborne, Mendel and Harris found that most of the albumin of a sodium chloride extract was precipitated by 45 percent saturation. Williams made some preliminary experiments with an aqueous extract of bean meal; he concluded that one third saturation with ammonium sulphate precipitates very little of the ricin, that one half saturation precipitates about nine-tenths of it and that two-thirds saturation precipitates very little more of the ricin than half saturation. Hence, the question arose if a sufficiently active preparation could not be obtained by half saturating an aqueous extract of the meal with ammonium sulphate, filtering or removing the excess of the ammonium sulphate solution by pressure and drying the precipitate.

Preparations of ricin obtained by filtration and drying the ammonium sulphate precipitates:

a. The two to one water extract from 50 grams bean meal, after filtration, was poured into an equal amount of saturated ammonium sulphate solution.

The resulting precipitate was then removed by filtration, washed with a small amount of water and dried in an oven at 47°. The yield was 0.563 grams (1.13 per cent). This agglutinated red blood cells, according to the method outlined above, at 1:4000. The lethal dose for white mice was 0.64 mg. per kilo (death within 48 hours).

b. Another preparation was made in the same way. The precipitate obtained was dissolved in distilled water and the insoluble globulins removed by filtration. The filtrate, containing some globulin and proteose in addition to the albumin, was again precipitated with ammonium sulphate and after filtration and drying in an oven at 47° yielded 0.962 grams from 50 grams of meal (1.92 percent). This preparation agglutinated red blood cells at 1:6000 and was lethal for white mice, by subcutaneous injection, in doses of 0.71 mg. per kilo, within 48 hours and in doses of 0.25 mgm. per kilo, within 72 hours.

c. Preparation of ricin obtained by filtration, pressing and drying the ammonium sulphate precipitate: Two hundred grams of meal were macerated with 400 cc water for a period of two hours. This mixture was then filtered under pressure; a filtrate of 356 cc was obtained. To this filtrate 350 cc of saturated

monium sulphate was added and the resulting precipitate was allowed to sediment for 12 hours. The supernatent liquid was removed by decantation and the precipitate by filtration under suction. The precipitate, which was a rather thick pasty mass, was transferred to a screw press where a further amount of the half saturated ammonium sulphate solution was pressed out. The press cake, after being dried in a vacuum dessicator, gave a yield of slightly more than 2 grams (about 1 percent).

This preparation was much darker than the previously prepared ammonium sulphate precipitates. It agglutinated at 1:14000 and white mice were killed by subcutaneous injections within 48 hours by doses of 0.25 milligram per kilo.

An exactly similar preparation was made at a somewhat later date which agglutinated red blood cells at 1:16000.

A third preparation of this type agglutinated at 1:14000 while a fourth preparation made by combining a number of pressed ammonium sulphate precipitates agglutinated at 1:13000.

It was thus seen that the most active preparation was obtained by half saturation of an aqueous extract of the bean meal with ammonium sulphate and re-

moving as much of the ammonium sulphate solution as possible from the precipitate by filtration and pressure; the yield of a highly active preparation was a little more than one precent. The precentage of ammonium sulphate in the preparation was undoubtedly high; it was not determined.

The preparation obtained in this manner is a dry, light gray, granular, amorphous powder which does not readily absorb water on exposure to the air. It is freely soluble in water with the exception of a small residue of insoluble globulin; this solution on evaporation leaves a dry easily powdered residue.

Our later preparations were made by adding, roughly, the amount of solid ammonium sulphate to the aqueous extract necessary to bring the latter to half saturation; in this way the volume of liquid which had to be handled was reduced. There is also reason to believe that the ricin is more completely precipitated in this manner, as it is contained in a smaller volume of liquid.

It would seem entirely feasible to prepare ricin on a large scale by a development of this method. The amount of ammonium sulphate required is shown by the following considerations: For each kilogram of bean meal used, 600 grams of ammonium sulphate (moist salt)

would be required (this to be added to the 2 liters of the 2:1 aqueous extract to make the latter half saturated). Almost all of this ammonium sulphate, however, could be recovered by evaporation and used again, as we have done in our laboratory experiments; for the only loss, aside from that resulting from the manipulations, is the negligible amount (much less than 10 Gm. per Kilo meal) which remains in the finished product.

(B) The Magnesium Sulphate Precipitation (cf. Stillmark, Cushny, Osborne and Mendel). We also used magnesium sulphate in a number of experiments for the purpose of salting out the proteins in castor bean meal. 66 grams of the salt added to 100 cc of the filtered macerate yielded 2.324 grams product per 100 grams of meal. This agglutinated at 1:3000 and was lethal to mice in doses of 0.37 milligrams per kilo in 4 days and 0.66 mg. in 3 days.

(C) Precipitation with Picric Acid: Picric acid, when added to the 2 to 1 filtrate obtained by macerating castor bean meal with water, causes an abundant precipitation, apparently of all the protein matter in solution; at least heat and nitric acid produce no further precipitate. This picric acid precipitate is very insoluble in water and may be repeatedly washed with only a slight loss of weight. However, the pre-

cipitated picrate is easily soluble in a dilute sodium bicarbonate solution.

The amount of picric acid necessary to produce a complete precipitation of the proteins in the 2 to 1 macerate has been determined somewhat roughly by adding a saturated picric acid solution to a known amount of this macerate until no further precipitation was observed. (Between each addition of picric acid the precipitate formed was allowed to sediment before additional acid was added). It was estimated from the above that nearly 1 gram of picric acid(78 cc of a saturated solution) per 100 cc of the macerate (from 50 gm. meal; or 20 grams picric acid per kilo meal) is required to complete the precipitation of the proteins. An average yield of this picrate has been 3.5 grams per 100 grams of meal.

The picrate may also be prepared by methods which obviate to some extent the handling of large volumes of liquid; this would be an advantage in large scale work. Thus, in some of the experiments, the calculated amount of solid picric acid (nearly 1 Gm. to each 100 cc of the 2:1 aqueous extract) was added, with constant stirring to the extract; in other experiments a saturated alcoholic solution of picric acid was added to the 2:1 aqueous extract in the proportion of 12 cc per

100 cc extract.

The precipitates formed by the above methods vary in color, depending apparently on the thoroughness with which they are washed; with little or no washing the color is a light, slightly orange yellow; when washed with several changes of water the color becomes darker and assumes a red to orange color. Some variation in the precipitates obtained at different times with picric acid has been observed aside from the color differences. Certain of these do not agglutinate red blood cells without the addition of small amounts of sodium bicarbonate; others produce agglutination without manipulation. The same variability also has been noted with preparations used in toxicity determinations, by subcutaneous injection, (white mice); this may be shown best by the following table:

TABLE IX.

Preparation	Agglutination Total	Not total	Lethal dose mg. per kilo	Time
Picrate I	1:2000 (1)	1:4000	0.64	2 days
" II	haemolysis	----	-----	-----
			1.0	2days
" III	1:4000	1:8000	0.50	3days
" II & III	1:1500	1:2000	1.0	4 "
Picrate IV (2)	---	1:200	0.50	2 "
" IV a	---	1:2	----	-----
" V	1:4000 (1)	1:6000	1.0	2 "
" 47			0.25	4 "

(1) Sodium bicarbonate added; No. V smaller amounts of the bicarbonate.

(2) IV_a Wash water from picrate IV.

Picrate III was injected subcutaneously into other animals with the following results:

TABLE X.

Animal	Dose per kilo	Result	Time
Guinea Pig	0.08 mg	death	67 hours
" "	0.25 "	"	40 "
" "	0.75 "	"	40 "
Rabbit	0.016"	alive after	7 days
"	0.05 "	death	40 hours
"	0.15 "	"	40 "
Cat	0.08 "	alive after	7 days
"	0.25 "	" "	7 "
"	0.75 "	death	21 hours
Mouse	0.33 "	alive after	7 days
"	0.50 "	death	3 "
"	1.00 "	"	2 "

These figures suggest that the picrate is one of the more active preparations of ricin. Although

it is less active than the ammonium sulphate precipitate it is about as active as the water extract (see below). A rather disappointing feature of the result is the relatively smaller yield. Nevertheless the picrate may present certain advantages over either the ammonium sulphate precipitate or the dried aqueous extract. It forms a very light, dry, powder which may easily be ground to an impalpable powder; in addition there is no tendency for it to absorb water. Caution should be exercised in grinding such preparations owing to the dust clouds which cause sneezing and lachrymation.

(D) Other experiments: In addition to the above experiments on precipitation a large number of others were performed; as the results are of little practical importance only brief reference will be made to them. (For other, not very practical methods see Dixson, Stillmark, Brieger, etc.)

a. Dialysis of the Aqueous Extract: The aqueous extract of bean meal contains, in addition to the posionous albumin, albumoses, inorganic salts and small amounts of globulin; the solution of the latter probably being facilitated by the inorganic salts present. It seemed possible that by dialyzing the aqueous extract some of the albumose and the inorganic salts might be removed. With the remval of the inorganic salts the globulin might be precipitated and removed by fil-

tration. The aqueous extract could then be evaporated or the ricin precipitated by ammonium sulphate. It was hoped in this way to secure a purer and more active preparation. Parchment dialyzing tubes of good quality were not available; they would moreover probably have the drawback of slow dialysis. Accordingly collodion dialyzing sacks made according to Kober were used. 100 cc extract of meal was dialyzed 4 days into 2900 cc of distilled water. Thymol was used as a preservative. The dialysate was filtered from a small amount of globulin and evaporated to dryness - yield 1.564 G. By analysis the water in the outer jar contained 1.974 G of material that had dialyzed. So 55% of the material in the water extract went through the membrane. In another experiment such dialyzed solutions lost roughly 20% of their agglutinating strength. Therefore it might be possible to purify somewhat a water extract by dialysis. But it would be a wasteful method.

b. Ethyl Alcohol: Some of the earliest efforts recorded in the literature (Dixson) to separate the poisonous constituent of castor beans were based upon its precipitation by alcohol. By careful work a number of experimenters have obtained active preparations in this manner (Cruz, Danysz, Truche and Alilaire, Agulhon, Assmann). The method consists in adding strong alcohol

to an aqueous, or sodium chloride, extract of the meal, filtering, washing with alcohol, or alcohol and ether, and drying; some have further purified it, and obtained a preparation leaving little ash, by redissolving in water and repeating the process. Cruz obtained in this way a preparation with less than 5 percent of ash.

Most writers (Dixson, Cushny and Osborne, Mendel and Harris, Assmann) are agreed that great care must be taken in this method, for alcohol rapidly renders the precipitate insoluble and non-toxic.

We made several preparations of ricin by modifications of the above method:

Preparation A, made by pouring the macerate into 96 percent ethyl alcohol; the precipitate was filtered under pressure, washed with alcohol which was then removed by suction, and the precipitate dried on a porous plate in vacuo.

Preparation K, made as above but washed with ether.

Preparation 28, made as above but washed with acetone; 5.364 gm. of dry powder was obtained from 100 gm. press cake.

Preparation 41, made from preparation 36 (an ammonium sulphate precipitate), which was dissolved in distilled water and filtered to remove insoluble globulins, by precipitating with ethyl alcohol; the pre-

cipitate was washed with alcohol and acetone and dried in vacuo.

The following preparations were made with methyl alcohol:

Preparation E made by pouring the macerate into methyl alcohol, washing with methyl alcohol, filtering with suction and drying in an oven at 45; the original nearly white powder became brown and brittle under this treatment.

Preparation J made from the macerate of 50 grams of the meal by pouring it into 2 volumes of methyl alcohol, washing the precipitate with methyl alcohol and ether, and drying in a vacuum dessicator; a yield of 1.733 grams was obtained.

These alcoholic precipitates were, generally speaking, rather active preparations as is shown by the following table:

TABLE XI.

Preparation		Agglutination Total	Agglutination Not total	Mouse, lethal dose per kilo	Time of death
Ethyl alcohol	A	1:3000	1:4000	0.29 mg.	3 days
" "	K	1:4000	1:6000	(0.36	7 "
				(0.62	2 "
				(0.41	3 "
				(0.50	3 "
				(0.30	4 "
				(0.53	4 "
				(0.30	4 "
				(0.40	5 "
" "	28	1:3000	1:5000	(0.25	2 "
				(1:00	2 "
				(0.50	3 "
" "	41	1:000	1:14000		
(Ammonium sulphate preparation					
	36	(1:14000	1:20000)		
Methyl alcohol	E	1:600	1:1000	(2.75	2 "
				(1.28	3 "
				(0.74	3 "
" "	J	1:1000	1:2000	(1.32	3 "
				(0.81	5 "
				(0.30	6 "
Acetone	29	1:2000	1:4000	(0.50	2 "
				(1.00	3 "
				(0.25	5 "

c. Acetone Precipitates: As will be noted, an "acetone" preparation of ricin is also included in the above table; this had generally about the same activity as the alcoholic precipitates. This was prepared as follows: the clear macerate was poured into two volumes of acetone; this caused the precipitation of a dense gummy mass. Upon decanting the mixture of water and acetone and treating this mass with additional acetone, it was more thoroughly dehydrated and could be easily powdered. One hundred grams of meal yielded 4.986 grams of the acetone preparation of ricin.

It may be concluded from the figures for agglutination and toxicity, as shown by the above table, that both ethyl alcohol and acetone yield a fairly toxic preparation which is about as active as the water extract and picrate but rather less active than the ammonium sulphate precipitate.

d. In addition to the above experiments on precipitation, a large number of others were performed, but the results are of so little practical importance that reference will be made to them only by the following outline:

TABLE XII.

Precipitant	Preparation	Average complete agglutination	Average lethal dose per kilo white mouse
Control	Water extract (1)	1:3000	0.50 mg.
Silver nitrate	20 filtrate	1:20	---
	20 ppt	1:2000	---
Alizarin sodium sulphonate	3 filtrate	1:20 incomplete	
	3 ppt.	1:1000	
Zinc chloride	22 filtrate	1:200	
	22 ppt. (1)	1:1000	
Phosphotungstic acid (2)	12 filtrate	1:20 incomplete	1.0 death
	12 ppt.	1:1000	(0.5) (0.33) alive
Metaphosphoric acid	11 filtrate	1:100	
Stannous Chloride	25 filtrate	1:100	
Methyl violet	2 filtrate	1:40	
Rosolic acid	4 filtrate	1:40	
Cochineal	5 filtrate	1:40	
Carbol fuchsiu	7 filtrate	1:20	
Lead acetate	21 filtrate	1:40	
Uranium nitrate	8 filtrate	1.20	
Ferric chloride	17 filtrate	1:20	

(1) These numbers are the laboratory numbers of the preparations.

(2) See also Woronzow.

In addition to the above, precipitates were

obtained with bichloride of mercury and with tannic acid, but in both cases these precipitates were entirely insoluble and did not agglutinate red blood cells; the filtrates were not tested.

3. Properties and Availability of the Crude Dried Extract: With the demonstration of the fact that water extracts ricin from the meal almost as well as does a salt solution and that in this way the amount of inert globulin extracted may be greatly diminished, the question arose whether, by the evaporation of an aqueous extract, a sufficiently toxic material could not be obtained without further treatment. Williams, working with a fresh bean, obtained 13.52 percent solid matter by extraction with water; he suggested that such an extract might be available for war purposes (see Section VIII). Williams stated hat this extract was fatal within 48 hours to rats in doses of 0.16 milligrams per kilo.

In the course of our investigation, a large number of water extracts have been made and it may be said, generally speaking, that a relatively highly toxic preparation of ricin may be obtained by the evaporation of the aqueous macerate to dryness.

The amount of dried extractive which can be obtained from 100 grams of meal varies with the degree to which the aqueous solvent is removed. By the use of

an air pump 80 to 90 percent of the solvent in a 2:1 mac maceration is recovered and the filtrate from this macerate has a solid content varying from about 4.5 percent to 5.5 percent. The recoverable amount of extractive, then, should be from 80 - 100 grams per kilo of meal. It is to be noted that this amount is somewhat under that reported by Williams as determined from a single experiment; our own figures are based upon more than 25 extractions of three different lots of meal.

The water extract, when dried, gives a hard resinous adhesive mass, which is difficult to remove from the drying trays. It dissolves on the addition of water, but there is usually a small amount of material which does not again go into solution. Such solutions may also be again dried and again form an adhesive varnish-like mass.

The activity of these extracts have been studied either by toxicity tests or, more generally, by agglutination. The agglutinating power has varied from 1:2000 to 1:3000 for complete agglutination; a few such extracts have agglutinated at 1:4000. Such figures, it may be recalled, are generally about the same as those obtained in agglutination tests on the weaker ammonium sulphate precipitated ricin preparations but only about one fourth that of the more active ones.

The toxicity of certain of these water

extracts for the white mouse has been determined.

TABLE XIII.

Agglutination and Toxicity to White Mice of Water Extracts.

Preparation	Total Agglutination	Toxicity Death in Days Doses in Mg. per Kg.							
		1	2	3	4	5	6	7-14	No death
1	1/2000	1.0		1.0				0.6	0.4
		0.5		0.5				0.5	0.4
				0.33				0.3	0.3
									0.027
2	1/3000	10.0			0.25				
		5.0							
		0.5							
		0.37							

It may be noted that this table shows very well the great individual susceptibility of different mice to this poison. In no case has it been possible, with any of our dried water extracts, to kill white mice with smaller doses than 0.25 milligrams per kilo, whereas we were able to do so with Williams preparation C made from the 1917 Hatian crop in doses of 0.13 milligrams per kilo.

STABILITY AND KEEPING PROPERTIES OF RICIN.

This is evidently a subject of very great importance in case large scale production is begun. From the fact that ricin is an albumin, or at least has many of the properties of an albumin, it was to be anticipated that it would be easily injured by heat and other physical agents which act upon proteins and that it would be subject to decomposition by bacteria and moulds; in thelatter case it might be desirable to add an antiseptic to its preparations. While it is true that ricin is, in general, injured by the same class of substances and by the same manipulations which are injurious to other albumins (a point which should always be remembered in dealing with the substance) it has, on the whole, proved more resistant than was to have been anticipated. Thus an active preparation has been prepared from seeds 30 years old (Kobert). The rather unexpected resistance shown by ricin to proteolytic enzymes has been alluded to already (Jacoby, Michaelis and Oppenheimer, Osborne, Mendel and Harris, Rochat, F. Muller, Kobert, Lau). Impure preparations were found by Jacoby to be not easily injured by hydrogen peroxide, whereas the toxicity of a purified preparation was destroyed. The toxicity was destroyed by flourescent substances in the presence of oxygen and light (Tappeiner, Jodlbauer) and to some extent in the dark (Busck). The subject of the action

of moulds and bacteria upon ricin has not been carefully investigated. It has, however, been noted that some moulds do not destroy its activity (Beneke, Kobert, Bokorney) and that solutions undergoing putrefaction (Cruz, and observations of our own), or exposed to the action of certain bacteria (Brieger), continue to be toxic. It may well be, however, that other moulds and bacteria would destroy its toxicity; hence a study of the action of antiseptics upon its keeping properties seemed desirable. This seemed especially important since Nicolle and Truche reported that the toxicity of a preparation preserved with glycerine had not undergone any diminution after 4 years and Levaditi and Mutermilch preserved a simple saline extract under toluene for more than 2 years without there being a loss of either toxic or agglutinating properties, whereas Field found a highly purified preparation to have lost its toxic, but not its agglutinating properties, after 2 and a half years (conditions under which it was kept not stated). Brieger, however, stated that a (somewhat crude) preparation retained its activity for 10 years.

Another point of much importance is the effect of heat upon toxicity. Ricin, like albumins in general, is readily coagulated by heat when in aqueous solution; the toxicity is destroyed at the same time.

It was chiefly on account of this fact that ricin was for a time considered to be an enzyme. The temperature at which the toxicity is destroyed apparently varies with the duration and manner of heating (Cushny, Osborne, Mendel and Harris) and the nature of the solution, it is frequently stated to be destroyed at 60° - 70° but higher figures have been given (Miessner and Rewald). Dry preparations withstand a higher temperature (Stillmark), Miessner and Rewald stated that such preparations were not destroyed at 120° but were between 125°-130°. It is evident that moist preparations should not be subjected to a temperature above 65° and this under some circumstances and with somewhat prolonged heating would be injurious.

The results of our own experience, like the observations, in general, of others, have demonstrated that ricin, at least as found in the relatively impure preparations with which we worked, is not very easily broken down. At the same time there is some evidence that it may be, under certain conditions, gradually decomposed, either by bacterial decomposition, for example, or by heat and through the action of certain preservations, as was the case with formaldehyde.

A number of tests were made upon solutions of preparations of ricin, not only at the time they were first made but also after a considerable lapse of time,

the solutions being kept at room temperature (about 25°). As was to be expected, mould and bacterial growth rapidly developed, but in spite of the very evident protein decomposition, such solutions retained their activity very nearly, if not entirely, unimpared. Nevertheless, as is evident, from Field's experiments, that, under some conditions decomposition does take place and suggests the possible value of using preservatives, were ricin to be prepared in large amounts.

Preservatives: Aside from the use of glycerine as a preservative (see above) and a remark by Levaditi and Mutermilch that they had kept a saline extract of the beans under toluene for two years without diminution in toxicity, we have found nothing in the literature on the effect of preservatives upon the keeping qualities and toxicity of ricin. A series of experiments accordingly were outlined with a view to following any progressive diminution in either the agglutinating or the toxic properties of ricin both with and without attempts at preservation with antiseptic substances.

Two preparations of ricin were made up into 1 percent solutions and a third preparation was placed, in measured amounts, on glass slides. This latter was accomplished by making a solution and placing aliquot parts of this upon the slides, after which the preparations were thoroughly dried. These dried preparations were

later, after a definite interval, prepared for testing by dissolving off the varnish-like film with a sufficient amount of 0.9 precent salt solution to give a 1:1000 solution.

The solutions and dried preparations were kept under the following conditions: (1) at about 25° (room temperature); (2) at 6° (cold room); (3) 9.0 percent sodium chloride; (4) 0.5 percent cresol, (5) 0.1 percent salicylic acid; (6) thymol, 1:1000; (7) formaldehyde, 0.2 percent. In all cases the preservatives were added to the solutions.

In each of the three series (two in solution and 1 the dried extract), there appears to have been a gradual decrease in the power to agglutinate red blood cells but very little or no decrease in the toxic property. An exception to this was the formaldehyde preserved solution which showed very rapid loss in both of the above properties of ricin. Also the dried preparations have retained their ricin-like activity with no certain impairment - certainly none which could be demonstrated by the tests which we used and which are approximations rather than absolute and quantitative. At all events, after a period of two months there is, very evidently, no serious impairment of the toxic properties of ricin even under the very unfavorable conditions of solutions kept in a warm room. The agglutinating

factor, on the other hand has manifestly undergone some change.

The above conclusions are based upon the data shown in the following tables:

TABLE XIV.

Preservation. 1 percent of Preparation K.

Table I.

Interval days	Preservative	Total Agglutination	Not total	Doses in Mg. per Kilo causing death						
				Days 2	3	4	5	6	7-14	Not Fatal 14 days
0	None	1/6000	1/8000							
3	"	1/6000	1/8000		0.66 0.5	0.3				
14	"	1/3000	1/4000							
28	"	1/2000	1/3000							0.5 0.33 0.25
42	"	----	1/1500							
60	"	1/1000	1/1500							

TABLE XV

Interval Days	Preservative	Total Agglutination	Not Total	Doses in Mg. per Kilo causing death						
				Days 2	3	4	5	6	7-14	Not Fatal 14 da.
0	Cold	1/4000	1/6000							
3	"	1/6000	1/8000		0.68	0.3		0.46		
14	"	1/3000	1/4000	0.52		0.25	0.37			
28	"	1/4000	1/5000							
42	"	1/2000	1/3000	2.5 1.0	0.5					
60	"	1/4000	1/5000							
0	Cresol	1/2000	1/4000							
3	"	1/5000	1/6000		0.66	0.29				0.37
14	"	1/3000	1/4000							
28	"	1/2000	1/3000					0.50		0.33 0.25
42	"	----	1/1500							
60	"	1/1400	1/2000							

TABLE XV. CONTINUED

Interval Days	Preservative	Total Agglutination	Not Total	Doses in Mg. per Kilo causing Death					
				Days 2	3	4	5	6	7-14
0	Thymol	1/4000	1/6000			0.53			
3	"	1/6000	1/8000		0.41	0.32			
14	"	1/3000	1/4000		0.25 0.50			0.38	
28	"	1/4000	1/5000						
42	"	1/2400	1/3000						
60	"	1/4000	1/5000						
0	9% Na Cl	1/6000	1/8000						
3	"	1/8000	----	0.62			0.4	0.26	
14	"	1/5000	1/6000		0.5			0.27 0.38	
28	"	1/5000	1/6000				0.5	0.33	0.25
42	"	1/4000	1/5000	1.0	0.5	0.33			
60	"	1/4000	1/5000						

TABLE XVI.

Interval days	Preservative	Total Agglutination	Not Total	Doses in Mg. per Kilo causing Death						
				Days 2	3	4	5	6	7-14	Not Fatal 14 da.
0	Formald.	1/4000	1/5000							
3	"	------	1/2000		1.5 2.5	0.6				
14	"	-------	1/2000			2.1				0.35 0.25

TABLE XVII.

Preservation 1 percent of Preparation C.

Interval Days	Preservative	Total Agglutination	Not Total	Doses in Mg. per Kilo causing Death						
				Days 2	3	4	5	6	7-14	Not Fatal 14 days
0	None	1/12000	1/16000		.62 .28	.15				
12	"	1/8000	1/10000							
35	"	1/4000	1/6000							
64	"	1/4000	1/5000	0.5						

TABLE XVII. Continued

Preservation 1 percent of Preparation C.

Interval days	Preservative	Total Agglutination	Not Total	Doses in Mg. per Kilo causing Death						
				Days 2	3	4	5	6	7-14	Not Fatal 14 da.
0	Cold	1/12000	1/16000							
12	"	1/8000	1/10000							
35	"	1/6000	1/8000							
54	"	1/8000	-----							
0	9% Na Cl	1/12000	1/16000							
12	"	1/8000	1/10000							
35	"	1/6000	1/8000	0.5 0.25		0.125				
54	"	1/5000	1/6000							
0	Thymol	1/10000	1/12000							
12	"	1/2000	1/8000							
35	"	1/6000	1/8000							
54	"	1/6000	1/8000							

TABLE XVIII

Interval days	Preservative	Total Agglutination	Not Total	Doses in Mg. per Kilo causing death						
				Days 2	3	4	5	6	7-14	Not fatal 14 days
0	Cresol	1/16000	----							
12	"	1/10000	1/12000							
35	"	1/4000	1/6000							
54	"	1/6000	1/8000							
0	Formal.	1/12000	1/16000							
12	"	1/2000	1/4000							
35	"	-----	1/2000							

TABLE XIX.

Preservation. Dry specimen of Preparation G.

Interval days	Preservative	Total Agglutination	Not Total	Doses in Mg. per Kilo causing death						
				Days 2	3	4	5	6	7-14	Not Fatal 14 days
0	None	1/4000	1/5000			0.54	0.59		0.3	
12	"	1/2400	1/3000							
24	"	1/2000	1/2400	1.0 0.5						0.33
35	"	1/1500	1/2000	1.0		0.5				
54	"	1/2400	1/3000	0.5		1.0				0.33
0	Cold	1/4000	1/5000							
12	"	1/2400	1/3000							
24	"	1/2000	1/2400	1.0		0.5			0.33	
35	"	1/1500	1/2000	1.0 0.5		0.33				
54	"	1/3000	----							
0	9% Na Cl	1/2000	1/4000							
12	"	1/2000	1/2400							
24	"	-----	1/2000				1.0		0.5	0.33
35	"	1/1000	1/1500	0.33		0.5 1.0				
54	"	1/2000	1/2400							

TABLE XX.

Interval days	Preservative	Total Agglutination	Not Total	Doses in Mg. per Kilo causing Death						
				Days 2	3	4	5	6	7-14	Not Fatal 14 days
0	Cresol	1/2000	1/4000						0.6 0.48 0.27	
12	"	1/2000	1/2400							
24	"	----	1/2000		1.0					0.5 0.33
35	"	1/5000	1/2000							
54	"	1/3000	----		1.0				0.5	0.33
0	(acid) Salicyl	1/2000	1/4000						0.67	0.42 0.30
12	"	1/2400	1/3000							
24	"	1/2000	1/2400							
36	"	1/1500	1/2000							
54	"	1/2400	1/3000							

TABLE XX Continued

Interval days	Preservative	Total Agglutination	Not Total	Doses in Mg. per Kilo causing death						
				Days 2	3	4	5	6	7-14	Not fatal 14 da.
0	None	1/2000	1/4000							
12	"	1/2400	1/3000							
24	"	1/2400	1/3000							
35	"	1/1500	1/2000							
54	"	1/3000	----							

The above results show that a dry preparation may be kept at ordinary room temperature at least 54 days without any diminution in toxicity; a preservative is not necessary. They also suggest that if an aqueous solution is to be kept for any considerable period, it should be kept in the cold or a preservative (such as cresol, thymol, or 9 percent sodium chloride) should be added.

Heat: The stability of ricin, in partly purified preparations, when subjected to the action of heat was studdied only within the range of certain well defined conditions, particularly since the results of others have suggested that the time factor was of much importance in the resistance of ricin to heat. Protein precipitation, in 1 percent solutions of ricin preparations has been noted at 55° but at this temperature both the agglutinating and the toxic factor remain intact. Our tests were all made upon 1 pecent solutions, heated for 30 minutes in test tubes submerged in a water bath. Under these conditions ricin begins to break down at about 75°, At 85° it is almost wholly decomposed. There seems to be a closely parallel destruction at the above temperatures of both the agglutinating and toxic factor. The results of our experiments on the stability of ricin to heat is shown in the following table :

TABLE XXI.

Temperature	Agglutination Total	Agglutination Not Total	Days till death and Dose 2	Days till death and Dose 3	Not dead 14 days
Control	1-10000	1-14000	0.5	0.125 0.25	
55°	1-10000	1-12000			
60°	1-10000	1-12000			
65°	1-10000	-----			
70°	1-10000	------	0.5	0.25	0.125
70°	1-10000	1-14000			
75°	1-10000	1-14000	0.5	0.125 0.25	
75°	1-4000	1-10000	0.5	0.25	0.125
80°	1-4000	1-6000	1.0	0.5	
85°	-----	1-1000			
90°	-----	1-200			

* Mice receiving doses of 2.5 - 1.0 - 0.5 have never shown any manifestations, or even a minor degree, of poisoning.

TOXICITY OF RICIN.

The toxicity of a given preparation of ricin depends upon a number of factors; e.g., how it is introduced into the body, the species of animal under experimentation, etc. Much also depends upon the character of the preparation.

(1) Purity and Character of the Preparation: Pure ricin has never been obtained; all preparations of it are known to contain other non-poisonous substances. The form in which ricin is present in the beans is unknown. There are indications that it is either in some form of combination which is less toxic or that there are present in the beans substances which diminish in some way its toxicity. This was shown by the following experiment of Williams: Twenty-eight mgm. per kilo of a given meal was buried under the skin of a rat; there was a distinct but by no means severe reaction, whereas an aqueous extract of only 1.3 mgm. of this meal, per kilo, sufficed to kill rats when injected subcutaneously. Such an experiment shows the desirability of using preparations from which as much of the foreign material as possible has been removed; this is probably more important when the poison is to be administered by some channels (for example by subcutaneous injection) than by others (by the mouth for example).

Extracts made in the same way from beans of different sources may have different degrees of toxicity. What determines this difference is not known. Williams, who examined seeds from different sources and which were of the crops of 1911, 1913, and 1917, concluded that the age of the bean was an important factor; he states "the difference in this respect is not very great in any case, though the beans of recent crops appear to be two or three times as toxic as the older ones", but the data he submits is not very convincing that the difference is as great as this. On the other hand from Williams' records there seemed to be as much difference between the toxicity of Haitian and Brazilian beans of the 1917 crops as between Indian beans of 1913 and 1917. Kobert found beans 30 years old to be toxic.

Whether the differences in the toxicity of extracts of beans of different sources and of different ages are only quantitative or not has not been determined; from what is known concerning ricin there is reason for thinking that there may be qualitative differences, i.e., that of preparations which would seems to be identical from their method of preparation etc. some may be more toxic than others. This, if true, is a matter of great practical importance, for in that case the toxicity of a given preparation could not be increased beyond a certain extent by further purification; in other words

ricin from one source may be inferior to that from another source. This will be clear from a brief discussion of the current views as to the nature of ricin.

So far we have simply called attention to the fact that the chemical reactions and elementary composition of ricin are those of a protein. As a poison it differs greatly from the better known organic poisons (alkaloids, etc.) and resembles closely the group of poisons (probably also protein) known as toxins, of which diphtheria toxin is one of the best examples. One of the most characteristic features of the toxins (including ricin) and one which distinguishes them sharply from all other poisons is that animals may be immunized against them and also that with certain of them (of which ricin is one) the blood serum of an immunized animal contains an antitoxin (in the case of ricin an "antiricin"). Another feature of certain of the toxins (ricin and diphtheria toxins for example), and one of great importance in this connection, is that they may apparently exist in two forms (Jacoby), a toxic and a relatively non-toxic form; these two forms are undistinguishable, by any known reaction except that one is toxic and the other non-toxic. The non-toxic form (commonly called toxoid) will, when administered to animals in non-fatal doses, even cause an absolutely

specific immunity and the formation of an absolutely specific antitoxin (see later). The toxic form may be converted into the non-toxic form by various agencies, chemical and physical (heat, for example), and non toxic ricin may be present in castor beans just as non-toxic diphtheria toxin is present in the cultures of diphtheria bacilli. It is not surprising, therefore, that castor beans vary in toxicity, without showing corresponding chemical differences.

Reference was made above to the experiments of Williams on beans from various sources; we had the opportunity of testing on mice a preparation from one of these beans (Haitian, 1917) made by Williams by extraction with water: it agglutinated red blood corpuscles at 1:6000 and was fatal to white mice within two days in doses of 0.25, 0.5 and 0.5 mgm. per kilo, and in four days in a dose of 0.125 mgm., although some mice recovered from 0.125 and 0,25 mgm. Similar extracts made from other beans agglutinated at 1:2000 - 3000 and were fatal to mice in 2 days in doses of 0.375-0.5 mgm. per kilo, in 3 days from doses of 0.33 - 0.5. Obviously the beans used by Williams in making this preparation were more active than most of those with which we worked. This was also in William's experiments, more toxic for rats than extracts made by similar methods from beans of other sources. There is therefore an apparent difference in

the toxicity dependent upon factors which cannot be determined other than to state that a given meal represents a particular variety or year's crop of the castor bean.

Finally, the difficulty in making any exact statement of toxicity is great since the toxicity experiments recorded in the literature have been made with a great variety of preparations and it is impossible to arrive at conclusions except on the basis of the same preparation. Moreover, since the fatal period varies from a few hours to weeks, and this depends to a considerable extent upon the dose (see later), it is always necessary to consider both the time element and the dose. It would be very fallacious for example, to compare two doses one of which caused death within 24 hours and the other not for a week. For this reason "average fatal doses" have little value unless the fatal period also is considered.

(2) Method of Administration: Ricin is toxic when introduced into the body by the mouth, as is shown by the large number of cases of poisoning in man (see later) and animals (Frohner) from eating castor beans, or the press cake, and by the results of the administration of preparations of ricin to animals; by the rectum (Tedeschi); by subcutaneous injection, the method most frequently employed in experimental work; by intramuscular injection (Field); by injection into veins, both systemic and portal

(Tedeschi); by injection into the peritoneal cavity (Bunting); by application to the cornea (Ehrlich, Cornevin, Cruz, Gebb; see Section VII.); by intracerebral injection (Rehns), probably by the respiratory tract (Rehns, Williams). In the present connection, only the administration of the poison by the subcutaneous, intramuscular, and intraperitoneal methods (the channels by which it might enter the body on missiles), and by the respiratory tract are of interest; the application to the conjunctiva might be of interest on account of the local effects. Of course it is not known how toxic ricin would be for man when administered by these methods, for in all of the recorded cases of poisoning in man the poison has been taken by the mouth. By comparing the effects on animals of the administration of the poison by these various methods, some conclusions may be drawn as to what the effects on man would probably be if it were similarly administered.

Practically all writers agree that in animals the poison is the least effective when given by mouth; (Stillmark, Ehrlich, Chatenay, Cruz, Kobert) although some of the most severe symptoms are of gastro-intestinal origin. Kobert remarked that 100 times as much was required by mouth as when given subcutaneously; accurate data however, are lacking. It is also not possible to even approximate the surely fatal dose of the

poison for man when taken by mouth, notwithstanding the rather numerous cases of poisoning reported. Differences between individuals and probably still more differences in the condition of the individuals (relation to the taking of food for example) at the time when the poison was taken seem to be extremely important; only in this way can such reports be explained according to which one or two beans (containing not more than 3-6 mg. ricin) has caused extremely severe poisoning whereas other individuals took a large number of the same beans without serious symptoms being produced (Bispham for example).

Ricin is a very powerful poison to man when it is taken by the mouth and there is no reason to doubt that it would be correspondingly more toxic when introduced subcutaneously etc. It would be very useful to know which animal man most resembles in susceptibility, but unfortunately this is not known. For present purposes, it would be safer to base doses for man upon those for the more resistant animals, although it is probable that he would prove more susceptible then they.

As to the relative toxicity of the poison when administered by different channels: writers are practically agreed that ricin kills in the smallest doses and in the shortest time when it is injected intra-

venously. Intramuscular injection seems to come second and intraperitoneal third; apparently the fatal dose by intravenous injection is about half of that when given intraperitoneally (Bunting). Nicolle and Cesari found about 2 1/2 times as much necessary to kill when given subcutaneously as by intravenous injection; Stepanoff reported that 6-7 times as much was required (rabbits). According to Ehrlich ricin may be absorbed from the conjunctiva in sufficient amount and sufficiently rapid to kill mice in 3 days. Small amounts applied to the conjunctiva are absorbed in sufficient amount to cause a high degree of immunity (Ehrlich, Gebb); there is first a period of general hypersuceptibility, or anaphylaxis, and then immunity (de Waele). Rehns stated that ricin introduced into the respiratory tract is fatal in the same doses and the same time as when given subcutaneously; his statements are not very convincing.

(3) Fatal Dose and Fatal Period: Numerous illustrations are to be found in the literature of how dependent the "fatal period" is upon the "fatal dose"; an amount of the poison which will not kill in a day or two, for example, may kill in a week or two. Great confusion has been brought into the literature on the toxicity of ricin by the failure of experimenters to state the "fatal period", the method of injection, the

weight of the animals, etc. A few statements found in the literature are quoted; it should be noted that the "ricin" was often prepared by different methods and the toxicity of the different preparation varied greatly. There is apparently, little or no relation between the size of the dose and the fatal period when maximum doses are injected, but there is a very distinct relation when smaller doses are used. The shortest fatal period which we observed (mice) was nearly 36 hours and this was with a dose which was 27 times the smallest dose which had produced death within the 48 hour period. This finding was based upon the following experiments; Preparation No= 26 (a water extract) was fatal within 41 hours after subcutaneous administration in doses of 0.375 and 0.50 mgm. per kilo and mice injected with the same preparation, in doses of 5.0 and 10.0 mg. per kilo, were alive and apparently not near death at the end of the first 24 hours, although both were found dead at the 40th hour.

The following are examples of the relation between the fatal dose and the fatal period to be found in the literature.

TABLE XXII.

de Waele

0.05 mgm. (subcut) fatal to mice (wt. not stated) in 60 hrs.)
0.1 " " " " " " " " " " 36 ")
5.0 " " " " rabbits " " " " 40 ")
10.0 " " " " " " " " " " 24 ")

Gebb

0.005 " " " to mice " " " "7-10 da.)
0.01 " " " " " " " " "4-6 ")
0.1-0.2 " " " " " " " " " 2 ")

Nicolle & Truche

0.01 "drop" not fatal to guinea pigs (wt. not stated)
0.1 " " " " " " " " " 3 da)
1.0 " " " " " " " " " 20 hrs)

Nicolle & Cesari

0.02 " (Intravenously) not fatal to guinea pig (wt. not stated)
0.02-0.09 " " fatal to guinea pig " " "1-11/2 da)
0.1-1.0 " " " " " " " " " few hrs)

Donald & Ungermann

1.5 mgm. " fatal to guinea pig " " "60-74 hrs)
5.0 " " " " " " " " " 21 ")

Durham

0.001 " (subcut) " to mice " " " 4 das.)
0.005 " " " " " " " " 2 ")
0.025 " " " " " " " " 2 ")

Figures analogous to the above have been obtained by us in numerous experiments; illustrations are to be found in various parts of this report.

No figures of this character are available for man; in the reported cases of death (to which we have had access) the fatal period has varied from 46 hours to 12 days or more. The poison was, in all these cases, taken by mouth; there is no reason to doubt that both the fatal period and the fatal dose would be correspondingly shorter and smaller in man if the ricin were introduced subcutaneously, intramuscularly, or intraperitonneally.

Closely connected with this problem of the relation of the fatal dose to the fatal period is that of the so-called "incubation period". Ricin, in animal experiments at least, does not act as a "rapid" poison; there is invariably what has been called an "incubation period", i.e., a period after the administration of the poison during which it seems to be having no effect. In this respect also it resembles the "toxins". This incubation period is most plainly seen when the poison is administered intravenously; there is a greater delay in the appearance of symptoms when the poison is administered by the mouth or subcutaneously. The delay in the latter cases is probably due to a considerable extent to slowness of absorption. The so-called incubation period after

intravenous injection may be due in part to the slowness with which the ricin leaves the blood (cf.A.Stepanoff, Brunner). This incubation period has not been thoroughly investigated. It varies with the species; de Waele stated that whereas the minimal fatal dose would kill a rabbit (subcutaneous injection) in 24 hours, the fatal period for a mouse was 3 days. There is no means of determining the incubation period for man for only poisoning by mouth is known and whatever the delay which occurs in the appearance of symptoms and death in such cases may be due largely to the slow absorption into the blood. The fact, however, that symptoms of poisoning do not usually (there are marked exceptions)appear for some hours after the poison has been taken suggests that there is here also an incubation period of some duration. The incubation period cannot be shortened beyond a certain extent by increasing the dose. Thus Cruz stated that 25 mgm. of a preparation, which ultimately killed a guinea pig in a fraction of a mgm. per kilo, did not kill in less than 13-17 hours (subcutaneous injection). Field found the minimal incubation period for intravenous injection into rabbits to be 5-6 hours; this was the same whether 10 or 100 mgm. were injected. Nicolle and Cesari, working with a very powerful solution of which 0.02 - 0.09 of a "drop" killed (intravenous injection) a guinea pig in 1-1 1/2 days and 0.1 - 1 drop in a few

hours, found that [illegible] by

hours, found that death was not produced more quickly by 3 drops than by 1 drop or less. We have recorded above an experiment in which the fatal period for a mouse was not greatly shortened by increasing the dose 27 times. De Waele found that Lecithin added to ricin shortened the incubation period; Dold and Ungermann state that lecithin will reduce the fatal period in quinea pigs (intravenous injection) from 21 to 9 hours. The latter experimenters also reported that, using smaller doses, the fatal period could be shortened from 60 to 70 hours when the ricin had been previously treated with the blood serum of the quinea pig. The above results suggest that possibly practical methods might be found for shortening the fatal period and so make ricin more useful in warfare. (The relation of lecithin to the fatal period of ricin has been further studied by Hanschmidt and Lawrow).

(4) Species Susceptibility: Lower organisms (paramoecia: Housmann and Koemer; algae and infusoria: Bokornyl) are little affected by ricin; the growth of bacteria does not seem to be affected by it (Cruz, ourselves). On the other hand, it is stated that the protoplasmic movements in some plants are inhibited by ricin (Kobert, who classes it ~~acts~~ as acting as a protoplasm poison) and indefinite statements to the effect that it is poisonous to insects have been made (Bokorney, Gullan).

All the vertebrates examined are very sensi- to the poison, but there are great variations in the degree of their susceptibility; fowls, among warm blooded animals, seem to be especially resistent, (Nagel, Cornevin) although geese seem to be very sensitive (Frőhner). (It is very toxic to tissue cultures according to Levaditi and Mutermilch; for action on the isolated frog heart see Kumagaiaud and Stillmark; for its action on muscle and nerve, Stillmark.

Data as to the variations of different animals species to ricin are very incomplete. Few of the numerous experiments reported on this subject are conclusive. Many factors contribute to this; few experimenters have reported many experiments with the same preparation; the time elapsing until death occurred is very often not given; few have required that the animals die within a definite time. A few of the unsatisfactory statements found in the literature may be stated. Ehrlich stated that it required about 35 mgm. of ricin to kill mice weighing 20 mg. when it was fed to them; the same preparation, when injected subcutaneously, was fatal to mice of the same weight in a dose of about 0.005 mgm. and much smaller doses killed guinea pigs. In contrast with this reported high resistance of mice to the oral administration of ricin is the statement of

Kobert that about 0.25 mgm. ricin per kilo is fatal to calves. Since death in man has resulted from the swallowing of 2 or 3 beans which would contain about 5-8 mgm. ricin it would seem that man should be classed as rather sensitive. Frőhner quotes (from Miessner) the following "average" fatal doses castor beans (time of death not stated) for various animals when given by mouth at once time:

TABLE XXIII

	Gm. per kilo.
Horse	0.1
Goose	0.4
Calf	0.5
Rabbit	1.0
Sheep	1.25
Hog	1.40
Beef	2.00
Pig	1.40
Goat	5.50
Fowl	14.00

" " "
ditto
"
"
"
"
"
"
"
"

Kobert placed, somewhat arbitrarily, the fatal dose of ricin for man, taken by mouth, at about 1 mgm. per kilo; men have died from much smaller and recovered from much larger doses (taken in the form of the bean).

The great resistance of frogs to ricin was also shown by Gushny, Lawrow and Osborne, Mendel and Harris. Field, working with a very highly purified preparation of ricin and administering it by intramuscular injection, gave the following fatal doses:

TABLE XXIV.

Rabbit	0.0001	mgm.	per	kilo	
Cat	0.0002	"	"	"	
Dog	0.0006	"	"	"	
Guinea Pig	0.0008	"	"	"	
Goat	0.003	"	"	"	(3 days)

Unfortunately Field does not give the fatal period except in the case of the goat; presumably his fatal dose was the smallest which eventually caused death, whereas the dose for the goat was probably larger than the minimal fatal dose in this sense.

Truche and Alilaire remark that ricin is very toxic for goats and mention an experiment in which 4 mgm. was fatal to a "large" goat.

Truche and Nicolle and Cesari stated that guinea pigs and mice are, per kilo, equally sensitive to ricin (intramuscular or subcutaneous injection), but the latter found that 100 times as much immune serum was necessary to protect mice as guinea pigs against ricin; other writers, however, are agreed in ascribing a much greater susceptibility to guinea pigs. Nicolle and Truche, using the same preparation, found "1 drop" to be fatal to rabbits (subcutaneous injection; weight of rabbit not stated) in 12 to 20 hours and 0.1 of a drop to cause death in a slightly longer period; 1 drop of ~~th~~ this killed a guinea pig (weight not stated) in 20 hours and 0.1 drop in 3 days. From such figures it would seem that per kilo animal ricin is more toxic to rabbits

tahn to guinea pigs, a conclusion in harmony with the work of others,) Field, Woronzow, Danysz). Woronzow, using a crude preparation, gave the following figures (method of administration and time of death, not stated)

TABLE XXV

Rabbit	0.03	mg.	per kilo.
Guinea pig	0.08	"	"
White rat	0.16	"	"
" mouse	0.40	"	"
Frog	2.00-4.00	"	"

The following table will show the results of our own work on the comparative resistance of different animals by subcutaneous injection.

TABLE XXVI.

	No.	$(NH_4)_2SO_4$	Water Extract	Picrate	Williams Water Extract
Mouse	231	0.5 - 0.25	2.5 - 0.33	1.0 -0.125	0.5
Rat	32	0.03- 0.005	0.2 -0.03		0.2 -0.05
Rabbit	6		0.5	0.15-0.05	
Guinea Pig	21	0.05-0.025	0.25-0.125	0.75-0.25	
Dog	2		0.5[x]		
Cat	5		0.5[x]	0.75	

[x]These figures are for 4 day deaths. When they were put into averages they were considered only in comparison to 4 day deaths in mice.

On the basis of averages from such figures and averages of minimal fatal and maximal non-fatal doses (which greatly overlap) we prepared the following approximate ratio of resistance of different animals. It making these averages the time interval before death was

always considered. To get really good figures of this kind not less than 100 animals of each kind should be used. We did not do so, so the figures may only be taken as tentative.

Ratio of resistance on the basis of the resistance of the mouse:

Mouse	100
Rat	12
Rabbit	12
Guinea Pig	60
Dog	200
Cat	200

(5) Method of Injection: A few experiments were made to determine whether subcutaneous; intramuscular; or intraperitoneal injections were more toxic. This work was done on mice, rats, and guinea pigs. The subcutaneous and intramuscular injections seem to run nearly parallel. The intraperitoneal injections were between two and three times more effective.

(6) Toxicity of Ricin for Man: The only data upon which any conjectures as to the toxicity of ricin for man can be based are derived from the cases of poisoning in which the castor oil beans have been eaten. In the records of cases accessible to us we find that death has been reported from the eating of the following number of seeds: 2,3,10,12 and 20. Castor oil seeds vary much in weight; assuming an average weight of 0.3 gm. per seed, the above number of seeds would have contained from about 0.006 to 0.06 mg. ricin.

On the other hand cases are reported in which recovery has followed the eating of a very large number of seeds even, perhaps, of a hundred. It is scarcely necessary to enumerate the conditions which may have prevented more serious symptoms from the larger numbers; the seeds may have been swallowed without being crushed by the teeth in which case the ricin would have been protected from solution and adsorption; many may have been eliminated by vomiting; if taken when processes of digestion were very active much of the ricin may have been destroyed by the digestive enzymes; the ricin may have been protected from solution and absorption by the globulin or fat; some beans may have contained more ricin than others. Furthermore, certain individuals may be especially resistent to the action of ricin just as certain animals are. On the other hand, it must be recognized that those who died from the smaller number of beans may have been unusually susceptible to the poison; hence it would be unsafe to take the smaller doses as they would ordinarily be fatal. On the whole, however, this kind of evidence would suggest that man belongs to the sensitive rather then to the resistant animals. This conclusion receives some support also from the fact that in cases of poisoning in man the symptoms frequently appar sooner than they do in most

animals, although in the latter case the poison may have been administered subcutaneously and in the former have been taken in an unfavorable form (the seed) for rapid action and by a channel (the digestive tract) favorable for the destruction of some of the poison.

From these considerations we are justified in concluding, for practical purposes, that man would be as sensitive to ricin when given subcutaneously as are other animals; but it would be better (as is done in this report) to base dosage upon that for the more resistant of the common animals.

(7) Toxicity of Ricin as a Dust: Rehns remarked that ricin, when introduced into the respiratory tract (whether in the form of dust or solution not stated) by pharyngeal-laryngeal catheterization, or puncture of the trachea, was as toxic, and the fatal period the same, as when it was introduced subcutaneously; no details of the experiments are given. The only other person who has investigated this subject, and his experiments were of a preliminary nature, was Williams. Williams endeavored to introduce ricin into the respiratory tract of rats in the form of a dust; the dust cloud was made by compression of a rubber bulb. Of eight rats receiving the toxin in this way, three died, with characteristic symptoms, in from three to fourteen days. As illustrating the defects in the method, it is to be noted that the effects

were by no means proportional to the amount of ricin which it was endeavored to suspend in the inspired air nor to the length of time the animals were exposed to the dust. Thus Williams estimated that one rat received only one tenth as much material as was received by another, yet the former died while the latter lived. Similarly, of three rats exposed to the, supposedly, same concentration of ricin, the one exposed for one minute died, whereas the two exposed for 3 and 4 minutes, respectively, lived. It is significant that one rat died eight days after inhaling not more than 0.65 mg. per kilo (0.1 mg. per 118 grams), a dose which indicates that ricin is able to produce death when inhaled in a relatively small dose. In the case of another rat, however, 6.5 mg. per kilo failed, by the same method, to produce any effects, except some sneezing.

In some of our experiments the ricin was suspended in the air in the form of a dust by means of intermittent blasts of air; in others by means of a revolving chamber, the animal(mouse) being placed in the center of the chamber. The results were disappointing and the details need notbe given here. The deaths were few even from (estimated) amounts of 5 to 10 mgm. of ricin per liter of air in the chamber, although occasionally much smaller amounts proved fatal. The results

were so disappointing as to raise the question whether the respiratory tract is capable of readily absorbing ricin. To secure information upon this question, a number of experiments were performed in which the ricin was introduced into the respiratory tract by means of a catheter in the trachea or the nares. The results were not striking; although 0.66 mgm. per kilo were at times fatal to rabbits, other animals recovered from larger doses. On the whole, a sufficient number of deaths occurred to suggest that the poison is absorbed in toxic amounts from the respiratory tract. Whether, however, this would prove a practical method of using ricin can probably only be determined by detonation tests and to secure the amounts of material necessary for these, ricin would have to be prepared on a larger scale than is feasible by laboratory methods.

SYMPTOMS AND PATHOLOGY OF RICIN POISONING.

Especial attention will be given in the following summary to the effects produced by ricin when the poison is injected subcutaneously or intramuscularly and also to the local effects (site of injection; eye), for these are of chief interest in the present connection.

(1) General Effects from the Subcutaneous etc. Injection of Ricin into Animals:

Mice show for the most part (aside from the local effects; see below) only a condition of depression, weakness, often inability to walk and diarrhoea, ruffled hair, loss of appetite and weight; the loss of we weight may amount to 25-40 percent if the animal survives for a week. Post mortem (see also Ehrlich, Gebb) there is usually a hemorrhagic condition of the intestine and of the body wall, but sometimes the condition is more "cholera-like" (Ehrlich) with rice-water-like contents; in this case the mucous membrane is pale and edematous. The spleen is dark red and soft; the adrenals hemorrhagic and congested. The pregnant uterus is congested with ecchymoses in the mucous membrane.

Rats: The symptoms and pathological changes in the rat are essentially the same as in the mouse.

Williams states that a rat exposed to a dust cloud of ricin immediately sneezed and rubbed its

snout with the fore-paws repeatedly; it sneezed frequently during the next four or five days and the mucus membrane of the nose was congested. The post mortem appearances in a rat which died on the third day after exposure to 5 mgm. of ricin in the form of a dust were described by Williams as follows: "The nose was full of mucus and blood exuded on slight pressure of the nostrils. Cyanosis was noted about and within the mouth and in all the toe nails. The eye-balls and sockets were greatly congested. The hair was noticeably loose, though the skin capillaries generally were very little congested. However, superficial congestion was marked over the top of the skull. The lungs were found to be extremely congested throughout and some bloody fluid was present in the pleural cavities. The liver was black; the spleen nearly normal in appearance; the small intestines slightly congested and covered with an orange color exudate in limited areas. The picture suggested that death was due primarily to the local effects of ricin in the lungs and secondarily to general absorption and circulation through the blood".

The symptoms of poisoning and the pathological changes may be followed in more detail in the larger laboratory animals; they vary somewhat with the size of the dose and the method of its administration.

Guinea Pigs: Ten to twelve hours after the

intravenous injection of a large dose of ricin (one causing death in about 24 hours) guinea pigs are depressed and show little inclination to move; when they do move they are very unsteady and may also show heightened reflexes; the hair is ruffled. Dyspnoea develops. According to Nicolle and Cesari the temperature falls progressively. The animals lie on their side, become comatose, but the coma is frequently interrupted by convulsive movements. Respiration becomes slow and finally ceases. On autopsy the abdominal organs are congested; the intestines show ecchymoses especially in Peyer's patches and are frequently distended with blood-stained liquid; the lungs and adrenal glands are frequently hyperaemic. The liver often has a marbled appearance. The pleural and peritoneal cavities often contain blood-stained liquid. After a large dose (death in about a day), given subcutaneously, there may be no symptoms for 16 to 20 hours; then depression and coma, with, in some cases, hyperexcitability, and finally death in coma. The pathological changes are similar to those described above; the retroperitoneal glands are enlarged and red and there may be necrotic areas in the liver; the lungs are congested and sometimes show areas of consolidation and the pleural cavity may contain fluid. With smaller doses and a longer fatal period almost the only symptom noted

is progressive weakness and depression. Diarrhoea seems not to occur in the guinea pig (Cruz). In chronic cases (death after about six weeks) the chief symptom is very marked emaciation. The liver is uniformly dark; this and the spleen and other organs show marked microscopical changes (described in detail by Flexner).

Rabbits:(cf. Müller, Flexner) when given a large dose of ricin subcutaneously show no symptoms for some (12 to 14) hours; they become quiet and refuse to eat. The onset of severe symptoms is rather sudden and consist, usually, of convulsions and opisthotonos followed in a few minutes by relaxation and apparent paralysis and then by a return of the convulsive effects; great prostration follows; dyspnoea develops and then death from respiratory paralysis. Shortly before death there is a marked fall of blood pressure (Müller). Frequently convulsions do not occur, the chief symptom is paralysis which begins somewhat suddenly. There is often profuse diarrhoea and cessation of urine secretion some hours before death. A marked decrease in body weight which can be accounted for only in part by increased nitrogen excretion has been described (Müller). Post mortem, punctiform hemorrhages scattered over the omentum and beneath the serous covering of the intestine and abdominal parietes are found; similar ecchymoses

are found beneath the serous covering of the solid abdominal organs and of the heart and lungs. There is usually an excess of fluid, often opaque and hemorrhagic, in the peritoneal cavity; the subserous hemorrhages are very numerous in the intestines. In the case of very rapid death the intestine is frequently filled with opaque almost fluid contents much resembling cholera stools. The intestines are always distended. In other cases the intestinal contents are tinged with blood and the general surface of the mucosa greatly congested and beset with pin-point hemorrhages; Peyer's patches are very prominent and deeply congested. The mucus membrane of the stomach is also congested; the spleen swollen, dark and soft. The liver is dark and hyperaemic and contains fecal lesions. The lymphatic glands, expecially the mesenteric glands, are swollen, softened and congested or hemorrhagic.

Cat: The symptoms in the cat resemble those of the rabbit. In 8 to 10 hours there is diarrhoea, often severe, and efforts at vomiting. The temperature is increased, at times to as high as 108° F. The cat lies in a corner without attempting to move; is weak and refuses to eat. The animal becomes gradually weaker and is unable to stand. Toward the end the temperature falls the cat appears to be very stupid; respiration is much

quickened and the pulse is very weak; later the respiration is slow. A condition of dyspnoea sets in and the animal dies apparently from respiratory failure.

Necropsy shows the lungs on both sides emphysematous with patches of consolidation. There is intense congestion of the omentum and intestines, the submucosa being particularly injected and marked haemorrhages into both adrenals. Small areas of haemorrhage are found on the parietal peritoneum. The spleen is ~~muc~~ much increased in size and very dark in color as is also the liver. The capsule of the kidneys strip easily, but there is no marked change apparent on section of the kidney.

Dogs: (cf. Foster) The most marked symptoms in dogs after a subcutaneous injection of ricin resemble those seen in acute infections; fever and prostration are especially marked, but there may be vomiting and diarrhoea. The symptoms appear in from 5-6 hours and consist in apathy, and depression followed by labored respiration and rapid irregular heart action; the nose is often hot and dry; the animal often has a chill; the temperature may rise to 105.6°F. With large but non-fatal doses the condition begins to improve after 4 or 5 days and recovery (aside from the local effect) is rapid. In case a minimal fatal dose has been given

the respiratory and cardiac symptoms become more pronounced; vomiting is frequent the animal refuses food; apathy and prostration increase and death may occur in 2 to 6 days.

At autopsy the abdominal cavity may contain dark blood-stained fluid; the omentum and mesentary are intensely congested and show areas of hemorrhage; the intestinal vessels are engorged. The intestine on being opened shows intense congestion from duodenum to rectum; from the ileocecal valve down there is bloody mucus; the mucosa is covered with ecchymoses. Small ecchymotic areas are often found on the epicardium especially along the coronariesl the lungs may show hypostatic congestion and numerous hemorrhagic areas. In one of our experiments on a bitch (subcutaneous injection into flank) death in 5 days, the mammary glands were greatly swollen, especially posteriorly, resembling huge abscesses, on section they showed marked congestion and gross hemorrhages.

Thus the outstanding feature of the gross pathological changes in ricin poisoning in animals is the condition of the intestine; the changes here are doubtless due largely to the fact that the poison, however, administered, is excreted into the intestine (Stepanoff, Cushny, Flexner).

For detailed studies on the microscopical

changes in the organs resulting from ricin poisoning see Flexner, Cruz, F. Müller, Berkley (brain) , Bunting ((bone marrow)).

(2) Special Symptoms:

Temperature: Flexner, who experimented with mice, guinea pigs and rabbits, and Foster, who worked with dogs, state that there is always a rise of temperature; the former stated that it averaged about 2° and the latter recorded temperatures up to 105.6°F. F. Müller (rabbits) found no change in temperature; Nicolle and Cesari (guinea pigs; large dose, quickly fatal) found only a progressive fall of temperature; Cruz (guinea pigs) found occasionally a slight elevation of temperature during the incubation period and a progressive fall (sometimes of 8° or 9° C. before death) after the symptoms developed. In our experiments on rabbits, cats, and dogs there was a rise of tempeature; the temperature of cats for example frequently rose to 106° - 107° F. There was a fall of temperature before death.

The urine of animals poisoned by ricin contains albumin, hyalin and epithelial casts and sometimes red blood corpuscles (Flexner, Cruz); the sulphur and nitrogen excretion is increased (F.Müller, Foster). Ricin especially after intravenous injection, causes first a diminution in the number of leucocytes and then, if the

animal lives, an increase (Stepanoff, F. Müller, Bunting). It has a destructive action on red blood corpuscles and leads to the appearance in the circulating blood of mucleated red corpuscles (Bunting).

(3) Local Effects: Ricin when injected subcutaneously into animals has a marked destructive action upon the tissues; this has been observed in many animals (mice; Ehrlich, Gebb; guinea pig: Cruz, Nicolle and Cesari: dog, Foster: goat: Truche; rabbit: Ehrlich and many others).

If the animal dies acutely, within 1-3 days, the local reaction may not be noticed on casual observation; but if the animal survives for several days the lesions are very marked; they develop more rapidly in some animals (guinea pigs for example) than in others (mice). In the guinea pig the local reaction develops about as follows: first day, slight edema; second and third day, more edema with numerous small ecchymoses in the surrounding tissues; fourth to sixth day, swelling diminishes, skin becomes tough, firm, and dry; seventh to tenth day, the dried skin falls off leaving an ulcer which exposes the muscle underneath; this often undergoes necrosis. A scab forms and the ulcer heals slowly by granulation. The ulcer, in our experience, appears a little later in the mouse and is usually very severe,

often involving the entire back and not healing for weeks.

These effects are, since they require considerable time for their full development, especially conspicuous after small non-fatal doses; they are very annoying in immunization experiments and often necessitate the abandonment, for considerable periods of time, of subcutaneous injections. A careful study of the doses necessary to cause serious local effects is very desirable; our own experiments indicate that in mice one tenth of the fatal dose usually causes severe ulceration. The importance of this from the offensive standpoint is obvious: every wound, however slight, made by a missile having only a fraction of a lethal dose would very probably make the man a casualty for a considerable period of time.

Ricin has a very injurious action upon the eye; this may prove of importance in the present connection. Ehrlich found that a 0.5 - 1 per cent solution applied to a mouse's eye caused intense congestion; stronger solutions caused a panophthalmitis and loss of the eye. Gebb made a number of observations on the effects of ricin upon the eyes of guinea pigs and rabbits incidental to his work on the immunization of these animals by the application of the poison to the eye; he stated that a solution of 1:1,000,000 caused slight

hyperemia, 1:10,000 reddening and swelling of the conjunctiva with the lids glued together on the following day; there were small hemorrhages but usually recovery in about six days. Some rabbits developed a purulent conjunctivitis (cf. also Valenti). De Waele found that when a small amount of ricin, which had little effect upon the first application to a rabbit's eye was applied to the same eye a second time about 15 hours later, a very severe reaction resulted; a condition of hypersusceptibility, or anaphylaxis, had developed.

We have not made as many observations upon this subject as should be done in view of its possible importance. Our animals have reacted somewhat unequally but this was to have been anticipated in view of the uncertain dosage of the poison when administered by the method we usually employed: dropping the powdered ricin directly into the eye. When the powdered ammonium sulphate precipitate or the dried aqueous extract (Section IV) was dropped upon the cornea of a rabbit's eye in amounts of 1 and 0.1 mgm (smaller amounts have not been tried) the only effects noted in the first four hours were slight lachrymation and reddening. The next morning (about 20 hours after the application) the effects were most severe: the eyelids were enormously swollen an and glued together; pus oozed from the slit. The conjunctiva was greatly swollen, congested and it was

difficult to open the lids sufficiently to see the eyeball clearly. The cornea was cloudy 24 hours later, the symptoms were about the same; the cornea was blood shot. One rabbit which had received 1 mgm. showed symptoms of systemic poisoning. In an experiment in which an aqueous solution was employed, the effects developed somewhat more rapidly; thus a drop of a solution, containing less than a mgm. of ricin, applied to the conjunctiva of a rabbit caused within 2 to 3 hours marked congestion, followed the next day by a purulent conjunctivitis with pus oozing from between the lids, and a clouding of the cornea; there was no improvement in ten days and the sight of the eye is probably permanently lost.

The eyes of the cat and guinea pig appear (but our experiments were few) to be less sensitive; however we have seen very severe symptoms develop in the eyes of both of those animals. Thus a milligram of the ammonium sulphate preparation dropped into the eye of a cat caused lachrymation and congestion; the lids were glued together in 2 or 3 hours and the next day a mucopurulent conjunctivitis had developed, which, however, cleared up rapidly so that the eye seemed almost normal after 3 days. Moderately severe effects were observed after 0.1 mgm. of the ammonium sulphate preparation had been applied to the eye.

We saw severe effects produced in the eye of a guinea pig from 0.1 mgm of the picrate compound of ricin; there was a blood-stained, muco-purulent discharge with much swelling and congestion; 1 mgm of the ammonium sulphate precipitate caused a more severe reaction (great swelling, haziness of the cornea etc).

(4) Poisoning by Ricin in Man: Stillmark and Beauvisage were able to find records of about 150 cases of castor bean poisoning in man (9 fatal) up to 1894; since that time a considerable number of additional cases with several deaths have been reported in the medical literature (for example: Bispham, Burroughs, Condos, Edson, Houze de l'Aulnoit, Hutchinson, Langenfeldt, Meldrum, Pecholier, Taylor, Vitrae, Wolfsolm, Wood). Many of these reports are not available in Boston and of the others many were very imperfectly reported; the following summary is only provisional.

Aside from a few somewhat vague reports of injury to the health and especially to the eyes among men working in factories with the press cake (Alilaire, Kobert) all of the recorded cases of poisoning seem to have resulted from the taking by mouth of castor oil beans. Such beans of course contain the castor oil and possibly some of the symptoms in some of the cases were partly due to the purgative action of this. But it is very improbable that the oil in such cases is ever an

important factor; for example purging, the only effect of the oil, is not a constant, although a usual, feature of the poisoning and it is of a different character from that caused by the oil; diarrhoea was not present in some of the cases in which the largest number of seeds were taken and in other cases it was a late symptom beginning only several days after the taking of the seeds; constipation for several days has been marked in some severe cases.

The symptoms of poisoning in man from eating castor oil beans are on the whole very similar to those caused by ricin in the lower animals. Perhaps the most constant symptom has been a condition of weakness or prostration; sometimes this has been practically the only symptom (Edson). This is also the most constant symptom of ricin poisoning in animals. Other symptoms observed in many cases in both man and animals are persistent vomiting (in animals which vomit) and nausea; diarrhoea with rice-water, sometimes bloody, evacuations, and tenderness and pain in the epigastric and umbilical regions and cramps in the limbs. These symptoms were followed by collapse; small, frequent, barely perceptible pulse and (in man) cold perspiration (in dog, a cold nose) and rapid respiration. Convulsions, which are usually seen in some animals(rabbits:

F. Müller) but not in others have also been reported in man. Icterus has also been reported in man and an analogous condition has been seen in animals. The changes in the urine noted in animals have been observed in man, but apparently they are usually not very pronounced in the latter. There has usually been some rise of temperature.

The fatal period (two but more frequently 5 to 12 days or more in man) is prolonged as it is in animals. Great individual variations in the resistance to the poison has been observed in man (s.g. by Bispham) as is so constantly seen in the lower animals.

The post mortem appearances, so far as they have been observed in man, are also similar to those found in animals: extreme congestion of the small intestines; stomach highly congested with small erosions, contents watery and bloodstained; meninges and brain congested with numerous petechiae; lungs congested (Meldrum).

Certain subjective symptoms are very common in man which cannot with certainty be attributed to animals; thus giddiness has been reported in many of the human cases; the lack of coordination in, and unsteadiness of, the movements of poisoned animals may be an analogous condition in the latter. Headache and griping pains in the abdomen are frequent symptoms.

The greatest difference between the symptoms seen in man and the lower animals is in the rapidity with which they often begin in the former. In animals the symptoms develop slowly, even on intravenous injection, whereas a number of cases are reported for man in which they began within a short time; thus a man is reported to have become very weak and collapsed "almost at once" from eating 1 bean (Gullan); a man "was overcome with giddiness to such an extent that he was unable to stand, and shortly after vomited eight times in rapid succession" after eating two seeds (Burroughs); one man ate 8 to 10 seeds, another 25 to 30 or more and both, about 45 minutes later, felt giddy and soon afterwards violent vomiting and purging commenced and 5 hours after eating the seeds one man was comatose and the other semicomatose (Hutchinson); more frequently however the symptoms have not commenced for two, three, four or more hours.

POSSIBLE USES OF RICIN IN WARFARE.

Two methods of using ricin in warfare suggest themselves: (1) as a poison to be applied to shrapnel bullets, (2) as dust clouds for its toxic action, if absorbed from the respiratory tract and for its action upon the eyes. The first of these was discussed in detail by Williams, who also reported a few preliminary experiments upon the second question.

(1) Use on Shrapnel Bullets: The results of Williams' work may be summarized as follows: A simple aqueous extract of an old sample of beans which still contained 27 percent of fat was prepared essentially according to the method outlined in Section IV. This was used in the following ways:

Sample 1. One portion of the filtrate was concentrated by distilling off the water in vacuo till the concentrate reached the consistency of a thin syrup. Two hundred shrapnel bullets were given a treatment with strong nitric acid for a few minutes to remove grease and roughen the surfaces. They were then thoroughly washed with tap water and covered with the syrupy concentrate. The dish containing the bullets and syrup was then placed in a vacuum dessicator over sulphuric acid to dry the material further. The bullets were stirred up at intervals of one to two hours in order to secure an

even coating of the material. As the concentration proceeded, the whole mass of bullets stuck together over night and had to be broken apart, leaving very rough surfaces. The concentrated material is extremely sticky and varnish-like so that a smooth hard and tough film may be secured on undisturbed surfaces.

Sample 2. A second portion of the filtrate described above was treated with 40 grams of moist finely ground ammonium sulphate per each 100 cc. The resulting precipitate was filtered off, dissolved in about 100 cc. of water and the solution dialysed for 48 hours in running water. The dialysate was then filtered and the filtrate to which 0.2 grams methyl orange was added was poured over 200 shrapnel bullets, previously cleaned with nitric acid. The bullets covered with liquid were then placed in a vacuum dessicator and treated as in the case of Sample 1. The methyl orange was used to render sample 2. distinguishable from 1. The comments made of Sample 1 apply also to Sample 2. The material in the second case, however, forms a somewhat more brittle film.

Sample 3. The third portion of the filtrate above described was treated precisely as was the material for Sample 2, except that the final solution was not evaporated on the bullets, but separately. The dried residue weighed 9.0 grams. It was pulverized and mixed.

200 shrapnel bullets were pitted by boring a hole with a 1/8" bit about one-third through the ball. About 30 mg. of the dry powdered ricin were placed in each hole and covered with a drop of glue. Two shrapnel shells were filled in the usual manner with a mixture of equal numberd of Samples 1, 2 and 3. A matrix of paraffin was used. The shells were filled under the supervision of Lieut. Kellogg, who has a record of details of procedure. These shells were fired into a wall constructed of composition board and lumber and filled with cotton waste. The bullets were collected after firing and separated into three types, the origin of every bullet except a few which were completely sheared being readily distinguished. The amount of ricin preparation on samples 1, 2, was determined by weighing three groups of 10 each of the coated bullets. The bullets were then washed free from adhering material and again weighed. A correction for adhering cotton waste, paraffin, etc., was made by filtering the decanted wash liquors and weighing the insoluble residue.

In case of sample 3, it was evident that no loss of ricin had occurred as the holes were still sealed with glue. Accordingly no estimation of ricin was necessary.

The wash liquors from each of these

samples after filtering were made up to known volumes and the solutions used for agglutination and toxicity tests. The material from 10 bullets of sample 3 was also removed and weighed; amounts were dissolved in water in order to perform similar experiments in this case."

The amount of crude ricin (Sample 1) which adhered to the bullets varied from 106.4 to 115.5 mgm. per bullet as determined on 3 groups of 10 each; the average was 112.2 mgm. The amount recovered from the bullets after firing varied from 68.3 to 72.6 mgm. per bullet, an average of 70.7 mgm; in other words, the bullets had retained 63 percent of this preparation of ricin. The toxic and agglutinating properties were not in the least diminished by the firing. The toxicity of the preparation used by Williams was low (the lowest of any with which he worked), but it was fatal to rats in 2 days in doses of about 0.3 mgm. per k. Should a man in proportion to his weight prove to be as susceptible to ricin as a rat, each of the bullets in the above experiment would have retained about 3 fatal doses.

Williams obtained the following results with Sample 2 (an ammonium sulphate precipitate, prepared from the same extract as Sample 1, which had been dissolved in water and dialyzed and the dialysate

evaporated on the bullets): An average of 15.1 mgm. adhered to each bullet; 10.5 mgm. remained after firing (a loss of 30 percent). The toxicity of the ricin on the fired bullets was the same as that on the unfired bullets. The fatal dose, in 2 days, for rate was about 0.1 mgm. per K.; this would correspond to about 7 mgm. for a man of 70 K. This preparation was fatal to rats in 15 days in about one half this dose; in other words, the bullets after firing had retained at least twice the amount which would probably have ultimately caused death in a man.

In the third set of bullets (Sample 3; the dialyzed ammonium precipitate which had been evaporated to dryness and placed in pits in the bullets and sealed in with glue), there was no loss on firing and the toxicity was not diminished. Williams reports no experiments as to whether the poison would easily be freed from these bullets in the subcutaneous or muscular tissues.

Regarding the use of paraffin as a matrix for the bullets Williams states: "The use of paraffin as a matrix from the standpoint of the preservation and utilization of the material seems to be well justified. Small amounts of paraffin are retained by the balls, but this was in no case found to be in the form of a film which could appreciably prevent solution and absorption in the body of the underlying soluble material. The paraffin coating is undoubtedly much less tenacious than

that of the ricin preparations. Its use would also in all probability serve a second good purpose, that of protecting the active material from the destructive action of air and light. It might on this account be well to immerse the bullets in paraffin as soon as the coating is sufficiently dry and keep them in this condition until ready to be loaded into shells. This would eliminate considerable mechanical loss of material in the handling necessary before and during the loading process and would further serve as an excellent protection to workmen engaged in shell filling".

These experiments show two important points: (1) easily prepared preparations of ricin can be made to adhere to shrapnel bullets, (2) there is no loss in toxicity of firing and even with the crudest method of coating the bullets, not a very considerable loss of the material itself.

There is another point which Williams does not discuss although it is shown by the protocells of his experiments; doses much below the fatal produce severe local reaction. In Section VII it was stated that even one tenth the fatal dose when injected subcutaneously has produced necroses and ulcers which healed very slowly. It is not unreasonable to suppose that every wound inflicted by a shrapnel bullet coated with ricin would produce a serious casualty, i.e., a casualty

much more severe than from the bullet without the ricin. Many wounds which would otherwise be trival would be fatal; bullets from which much of the ricin had been dislodged would probably still retain enough of the poison to make even slight wounds severe and protracted. (An important point in this connection which needs investigation is the effect of ordinary surgical treatment upon such wounds; it would seem improbable that treatment under field conditions would materially affect either the general or local effects of the poison).

We have described in Section IV three commercially available preparations which might be used in this connection: (1) the crude aqueous extract, such as Williams used, (2) the ammonium sulphate precipitate, and (3) the picrate, of these the aqueous extract has the advantage of readily adhering to the bullets without the addition of any other adhesive material; however it might be found in practice more feasible to start with dry easily powdered materials like the ammonium sulphate precipitate or the picrate and add an adhesive material like dextrin. The picrate would probably have the disadvantage of not producing symptoms as rapidly as the others on account of its relative insolubility; on the other hand the latter property and the fact that it is not hydroscopic might, under some conditions, be

advantageous. The ammonium sulphate precipitate has the advantage of greater toxicity than the others. Moreover, some laboratory experiments which we made suggest that it will adhere to bullets fairly well when simply moistened with water and dried on the bullets; in any case, there would probably be no difficulty in getting it to adhere to bullets by simple, practical methods.

In endeavoring to estimate the probable available supply of material for applying to shrapnel bullets, it would be well to count on using about the following amounts of each of the above preparations on each bullet; the estimated fatal doses for man are based upon those for the more resistant animals needed to cause death within 2 to 4 days.

TABLE XXVII.

Dried aqueous extract	Estimated fatal dose	Amt. on each bullet
	15 - 30 mgm.	100 mgm.
Ammonium sulphate precipitate	3.5 - 10 "	15 "
Picrate	30 - 50 "	100 "

As will be shown in Section IX the annual productions of press cake (almost wholly from imported seeds) in the United States has been from 10 to 15 thousand tons. It was anticipated in the spring of 1918 that there would be grown in the United States this year 2,000,000 bushels of the seeds; this would yield about 25000 tons of the press cake. From 1 ton of the press

cake there can be obtained about 90 kilos of the simple aqueous extract, 9 kilos of the ammonium sulphate precipitate or 32 kilos of the picrate. The available supply of raw material would, accordingly, yield about 40 to 75 billion fatal doses of the aqueous extract, 20 to 25 billion of the ammonium sulphate precipitate or 15 to 25 billion of the picrate. Assuming that it would be desirable, in order to make allowances for wastage and to secure more prompt effects, to place from three to five surely fatal doses upon each bullet there would be available material to coat bullets as follows:

TABLE XXVIII.

Aqueous extract	15,000,000,000	-	25,000,000,000
Ammonium sulphate preparation	5,000,000,000	-	10,000,000,000
Picrate	4,000,000,000	-	10,000,000,000

This would allow of a wastage of at least two-thirds of the material.

(2) Use as Dust Cloud:

a. Toxic Action Through the Respiratory Tract. As was explained in Section VI, our results did not seem to indicate that ricin is excessively toxic when introduced by the respiratory tract; our methods, however, were not such as would give a decisive answer to this question. Experiments on this subject should be carried out under field conditions but to do this it

would be necessary to prepare the material on a semi-large scale.

Ricin is very toxic for man when taken by the mouth (Section VII); in a dense cloud of ricin the possibility of absorption of sufficient ricin from the mouth and with the swallowed saliva as well as from the respiratory tract and eye, to cause poisoning should be considered.

b. Injurious Action on the Eye. It has been shown in Section VI that 0.1mgm. of available ricin preparations applied to the eye as a powder would cause most severe injury to the eye of a sensitive animal; in many cases such an amount seems to permanently impair vision. 1 mgm. similarly applied caused severe symptoms, with loss of use of the eye for a few days, in more resistent animals. The sensitiveness of the human eye to ricin is not known, but it is most probable that 1 mgm. would cause very severe effects. One hundred grams, the amount which could easily be placed in a shell, would be capable of injuring 100,000 and very probably 1,000,000 eyes. How great the chances would be of the necessary amount reaching an eye when such a shell was exploded, we have no means of knowing; a person would be inclinad to guess that a shrapnel shell containing such an amount of ricin would be as dangerous to the eyes(on

account of the ricin) as to the body as a whole on account of the bullets. In other words, the casualties from a shrapnel shell might be doubled. If, in addition, the shrapnel bullets were coated with ricin, the effectiveness of these shells would be very materially increased. Then also the distinct probability that casualties would result from the inhalation of the dust should be considered.

Experimentation would be necessary to determine which of the three ricin preparations described in Section IV would be most suitable for this purpose; the ammonium sulphate and picrate preparations, not being hydroscopic, would doubtless remain in the air longer as a dust. But the dried aqueous extract, being hydroscopic, would adhere to the clothes and equipment and might be subsequently carried to the eyes.

The material available would suffice for placing 100 gram quantities of these preparations in from 2 to 20 million shells.

THE CASTOR OIL INDUSTRY.

The following data were collected by ~~Willi~~ Williams in the spring of 1918.

(1) Sources of Beans:

Practically all cast or oil produced in the United States is made from imported seeds. At one time,

a considerable quantity of the beans raised, especially in Kansas and Oklahoma, but the crop has ceased to be popular. However, a general effort has been made to stimulate production in this country so that possibly as much as two million bushels may be harvested in 1918. The Air Craft Production Board, however, now seems to regard the use of caster oil for lubrication of aeroplane motors less favorably than the experts of our European allies and this increase may accordingly fail to materialize. However, the industry, as well as the government, is disposed to stimulate nearby production on account of the present shipping conditions, as in the past a large proportion of imported beans have come from India, involving a long transport by sea.

The following figures on importations of castor beans into the United States are taken from the Oil, Paint and Drug Reporter Annual Review, Feb.27,1918.

Fiscal Year	Bushels of 50# each[x]
1912-13	824,574
1913-14	1,043,928
1914-15	924,605
1915-16	1,071,969
1916-17	767,075

About 80 percent of these importations were from India. This percentage may be expected to show a decrease in favor of West India and South American sources during 1917-18 on account of the shortage of shipping and

government control of British ships. Haiti is ex[illegible] to furnich a considerable amount during the curr[illegible] as the government has taken steps to assure supplies from that source. For further date on production see the Oil, Paint and Drug Reporter, November 12th and December 10th,1917.

(2) Methods of Milling:

The entire importation of beans is expressed in the United States for the oil and cake. Castor Oil is used for leather substitutes, turkey red oil, tanglefoot fly paper, soap and for medicinal purposes. The beans yield about 45 percent of oil in commerical practice. The cake, consisting roughly of 50 percent of the weight of the beans, is used as fertilizer and sells in the neighborhood of $35.00 a ton. The annual production of cake in the United States is from 10,000 to 15,000 tons.

The procedure followed in the castor oil mills is to press first at a low temperature, about 40°C. By this means a domestic bushel of 46 lbs., customarily yields about 16 lbs. of so-called "cold-drawn" oil which is regarded as of a superior grade, especially for aeroplane lubrication and for medicinal uses. An additional 6 or 7 lbs. of darker oil is removed by subsequent treatment. This consists of subjection to live steam and a hot pressing followed by extraction with solvent

naptha. Or in some mills, the hot pressing is omitted and the cake is extracted with solvent naptha directly after steaming.

(3) Availability of Cake:

Fortunately for the purposes contemplated by the present investigation, the castor oil manufacturing business is largely in the hands of a few concerns. The following firms are said to produce about 90 percent of the total for the United States:

Baker Castor OilCo.,120 Broadway, New York City,N.Y.
Spencer Kellogg & Co.,111 Broadway, New York,City,N.Y.
Toledo Seed & Oil Co., Toledo, Ohio.
O & W.Thumm Co., Grand Rapids, Michigan.

The concentration of the business in these hands would obviously facilitate contracting for the large quantity of suitable cake, which may involve some modification of current practice.

IMMUNITY TO RICIN; ANTIRICIN.

Should ricin be prepared on a large scale or should it be used in warfare, the subject of immunity to this substance would become of great importance, for ricin is one of the few poisons for which, in animal experiments, an extremely efficient antidote is known; it is possible to obtain an antitoxin to ricin equal to those for diphtheria and tetanus. Hence, although we have made but few experiments ourselves upon this subject, a brief summary of what has been done may be of use.

When non-fatal doses of ricin are administered to animals or, better, when several such doses are given in increasing amounts, the animals rapidly acquire a marked immunity to the poison so that they will survive hundreds and even thousands of times the dose which would ordinarily be fatal. A considerable degree of immunity results from a single injection of ricin. Mice, rabbits, guinea-pigs, goats, swine and dogs have been immunized in this manner. The ricin, given to produce the immunity, may be administered by the mouth, subcutaneously, intraperitoneally (Tedeschi) or applied to the conjunctiva (Ehrlich, Gebb). Most frequently it has been given first by the mouth and then by subcutaneous injection (Ehrlich, Cornevin, Raubitschek, Truche). Immunity may also be produced by the injection of ricin, the toxic action of which has been destroyed by heating to 100°C; 2 or 3 injections at 8 day intervals are said to suffice (Cornevin). Perhaps the greatest degree of immunity obtained is in the case of rabbits which have survived the administration of an amount of the poison which would be fatal to 5000 untreated rabbits (Ehrlich, Cushny). The immune animals will not only survive many times the fatal dose but their eyes are no longer affected when the poison is applied to them and the local reactions and necrosis is far less when the poison is injected subcu-

taneously (Foster, Ehrlich). How long the immunity continues has not been determined; it is known, however, to last for many months. (For discussion of other features of immunity to ricin see Ehrlich, Truche and Alilaire, Watson, Jacoby, Madsen, and Walbum, Danysz, Loewenstein).

The blood serum of immunized animals contains an antitoxin which when injected into other animals protects against all of the toxic effects of ricin (Ehrlich, Stepanoff, Jacoby, Danysz, Madsen and Walbum, Fraenkel, Gebb, Truche, Nicolle and Cesari). Thus Ehrlich found that after the subcutaneous injection of a small amount of immune rabbit serum into a mouse, the latter survived at least 1300 fatal doses of ricin; Gebb found that 0.5 cc. of the serum of a rabbit which had been immunized by the instillation of ricin into the eye protected mice against 500 fatal doses; Truche found that 0.2 cc. of immune goat serum protected mice against 500 fatal doses; Danysz found that 1 cc. of immune goat serum neutralized 1000 fatal doses for a guinea pig. The local lesions from subcutaneous injection of the ricin are also prevented or greatly diminished in intensity (Truche) and also the eye symptoms from direct application. The protection against ricin afforded by a subcutaneous injection of antiricin was still marked

after 24 days (Ehrlich). Antiricin injected intravenously was present in the blood 24 hours later but absent after a week (Stepanoff).

The antitoxin maintains its activity for a long time (Fraenkel); it can also be prepared in a concentrated form analogous to similar preparations of diphtheria and tetanus antitoxins (Jacoby). Roemer, some years ago, prepared on a commercial scale an antitoxin to abrin (a toxalbumin very similar to ricin) for use in checking the action of this poison on the eye; antiricin could undoubtedly be similarly prepared on a commercial scale for the protection of workmen against ricin.

The antiricin not only prevents the toxic action of ricin but it prevents the agglutination of blood corpuscles (Ehrlich, Danysz, Jacoby, Madsen and Walbum). Fraenkel found that 0.08 cc. of immune goat serum prevented the agglutinating action of 5 milligrams of an active preparation of ricin. Upon this anti-agglutinating action is based what seems to be the most trustworthy test for ricin (the complement fixation test of Bierbaum). The process of immunization and the degree of immunity attained may be followed by testing the anti-agglutinating action of the serum of the animal undergoing immunization (Gebb and others). It is an interest-

ing fact that the red blood corpuscles of an immune animal are not resistent to the agglutinating action of ricin. (Cushny, Jacoby, Loewenstein, F. Müller, Miessner and Rewald).

The antiricin serum also contains precipitins, i.e., substances which cause a precipitate to form when added to solutions containing ricin (Loewenstein, Jacoby, Madsen and Walbum and others); upon this reaction is based another method for detecting ricin (Miessner, Mooser, W. Müller). Since, however, ricin alone when added to some blood sera (Stillmark, Lau, Kraus, Durham, Cruz, Wilenko, Michaelis and Steindorff) and other proteins, causes a precipitate (although this is less marked than that caused by the immune serum) the precipitin method for detecting ricin is not considered as good as other methods (Bierbaum).

Hypersusceptibility; Anaphylaxis. In the beginning of the immunization of animals to ricin there is a period during which they are hypersusceptibile to the poison (anaphylaxis); the time of the appearance and the duration of this period varies with different species (de Waele). Man may also become hypersensitive and this condition may be permanent; exposure to ricin causes in such persons symptoms analogous to those of hay fever and urticaria may result from its direct application to

the skin. It is said that men employed in factories where castor beans are worked up may acquire a similar hypersusceptibility. (Alilaire).

BIBLIOGRAPHY

Agulhon, H. -Etudes sur la Ricine. Recherche de la Ricine (toxine et agglutinine) dans les differentes especes et varietés de Ricin.-Annal. Institut Pasteur, 1914, 28, p. 819.

Agulhon, H.-Etudes sur la ricine (Toxine et agglutinine) pendant la germination des graines de ricin. -Annal. Inst. Pasteur, 1915, 29, p. 237.

Alilaire, E.-Etudes sur la ricine. III - Hypersensibilite a la ricine. Annal. Inst. Pasteur, 1914, 28,p.605.

Assmann, F.-Beiträge zur Kenntniss pflanzlicher Agglutinine. Arch. f.d. ges. Physiol., 1911,137, p.489.

Baroni, V. and Jonesco-Mihaiesti, C.-Sur la destruction par les rayons ultra-violets des principes actifs des serums normaux et prepares.-Compt. rend. soc. biol. 1910, 68,p.

Baumgarten, P.-Mikroskopische Untersuchungen uber Hamolyse im heterogenen Serum.-Berl. kl. Wochensch. 1901.

Beauvisage.-(1) Danger des tourteaux de ricin. Lyon, 1893. (2) Toxicite des grains de ricin, Paris 1894.

Beneke, F.-Ueber die giftige wirkung des

Ricinuskuchen.- Zeitsch. d. allgens. Öster, Apothekervereins, 1887, 25, p. 421.

Berkley, H.J.-Ricin poisoning. Experimental lesions produced by the action of ricin on the cortical nerve cell of the guinea pig's and rabbit's brain.- The Johns Hopkins Hospital Reports, 1896, 6, p.55.

Berkley, H.J.-Experimental lesions produced by the action of ricin on the cortical nerve cell of the guinea pig's and rabbit's brain. Med. Record, 1897, 49, p. 325.

Bierbaum, K.- Beitrag zur Giftigkeit des Semen ricin communis 1906.

Bierbaum, K.-Der Nachweis von Bestandteilen des Rizinussamens in Futtermitteln mit Hilfe der Komplementablenkunzsmethode.- Zeitsch. f. Infektionsk. d. Haustiere 1912, 12,p. 351.

Bispham, W. N.- Report of cases of poisoning by fruit of Ricinus communis.-Amer, Jour. Med. Sciences, 1903, 126, p. 319.

Bispham, W. N. - Poisoning by the castor oil bean. Medical News, 1903, 82, p. 809.

Bokorny, Th.- Vergleichende Studien über die Giftwirkung verschiedener chemischer Substanzen bei Algen und Infusorien.- Arch. f.d. ges Physiol., 1896, 64, p. 262.

Brieger, L.-Versuche zur Reinigung des Ricins etc.-Festschrift zum 60 Geburtstage von R. Koch. 1903, p. 445.

Brunner, G.-Recherches sur l'action des poisons bacteriens et vegetaux.- Arch. d. sci. biol. 1898, 6, p. 189.

Bunting, C.H.-Experimental Anaemias in the Rabbit.-Jour. exp. Med. 1906, 8, p. 625.

Bunting, C.H.-Experimental Anemias.- Jour. Amer. Med. Assoc. 1907, 49, p. 476.

Burroughs, W. J. -Poisonous effects on Ricinus communis.- Brit. Med. Jour. 1903, ii, p. 836.

Busck, G.-Die photobiologischen Sensibilisatoren and ihre Eiweissverbindungen.-Biochem. Zeitsch, 1906,1, p. 425.

Calloud.-Journ. de Pharm. et de Chim. 1848 p. 189.

Chatenay,G. Les reactions leucocytaires vis-vis de certaines toxines vegetales et animales. Paris, 1894.

Collin, E. Tourteau de ricin ses dangers, ses caracteres anatomiques.-Journ. de Pharm. et de Chim. 1903, (6), 17, pp. 361-422.

Condos, E.A case of poisonous symptoms from castor seed confection; recovery.- Indian Med. Rec. 1895 9, p. 47.

Cornevin, Ch.-Procede de vaccination contre l'ennpoisonnement par le ricin. Introduction consecutive des graines et dea tourteaux de ricin dans la ration des animaux.-Compt. rend. de l'acad. des sciences, 1897, 124 p.835.

Cornevin, Ch.-Journ. de Lyon, 1897, p.25.

Cornevin, Ch.- Ann agron. 1898, 23,p.289.

Cruz, G.-Les alterations histologiques dans l'empoisonnement par la ricine. Arch. de Med. exp. et. d. anat. pathol. 1899,11,p.238.

Cruz, G.-Etude toxicologique de la ricine. Ann. d'hyg. pub. et de med. leg. 1895.(3) 40,p.344.

Cushny, A.R.-Ueber das Ricinusgift.-Arch. exp. Path. u. Pharmakol,1898, 41,p. 439.

Danysz, J.-Contributions a l'etude des proprietes et de la nature des melanges des toxines avec leur anti-toxines.-Ann. Inst. Pasteur, 1902, 16,p. 331.

Dixson.T.-Ricinus communis.-Austral. Med. Gaz. 1887, 6,pp.137,155.

Dixson,T.-On the active principles of castor oil.-Med. Chir. Trans.,London.1887,70,p.107

Dixson,T.-Ricinus communis.-Proc. Roy. Med. Chir. Soc. London. 1885-6,ii,152.

Dold, H. and Ungermann, E.-Beiträge zum Mechanismus der Toxinwirkung.-Zeitsch. f. Immunitätsforsch 1911, 11,p, 86.

Durham, H.E.-Einige Studien über Abrus- and Rizinus-Samen.-Arch.f. Hygiene, 1913, 81,p.273.

Durham, H.E.-Some theoretical considerations upon the nature of Ahhlutinins etc.-Jour. of exp. Med. 1901, 5,p.350.

Edson, B.- Notes on sixteen cases of poisoning by the castor oil bean.- Brooklyn Med. Jour. 1888, 1,p.131.

Ehrlich,P.-Experimentelle Untersuchungen über Immunität.- Deut. med. Wochensch. 1891, 17, I Ueber Ricin. p.976; Ueber Abrin,p.1218.

Ehrlich, P.-Zur Kenntniss der Antitoxinwirkung.-Fortschr. d. Med. 1897,15,p.41.

Ehrlich, P.-Ueber Immunität durch Vererbung and Saugung.-Zeitsch.f. Hygiene u. Infektionskrankheit, 1892, 12, 183.

Ehrlich, P.-Schlussbetrachtungen.Nothnagels spez. Pathol. u. Ther.8,1901.

Ehrlich,P.-Die Werthemessung d. Diphtheriheilserums, etc.-Klin. Jahrb. 1897, p.20.

v. Eisler. M.-Ist die haemagglutination und Haemolyse, durch Ricin und Haemolysin hervorgerufen, eine Säurewirkung?-Gentrbll. f. Bakt. Abt. I, Orig. 1908 46,p.353.

v. Eisler, M. and Portheim, L.

Zeitsch.f.Immunitätsforsch. 1908,1,151.

v. Eisler, M. and Portheim, L.-Ueber Haemagglutinine in Pflanzen. Ber. d. deutsch. botan. Ges. 1911,29,p.419.

v. Eisler, M. and Tsuru, J.-Ueber Beeinflussung der Agglutinierbarkeit roter Blutkörperchen durch verschiedene haemolytische Gifte.-Zeitsch.f. Immunitätsforsch. Orig. 1910,6,p.305.

Elfstrand, M.-Ueber giftige Eiweisse, welche Blutkörperchen verkleben. Upsala. 1897.

Elfstrand, M.-Ueber blutkorperchenagglutinierende Eiweisse. Görbersdorfer Veröffent., berausgegeben von Kobert, 1898, 1 p. 11.

Field, C.W.-Agglutinating action of ricin on red blood corpuscles in isotonic sugar.-Proc. N.Y. Path. Soc. 1908-9, 8, p.100.

Field, C.W.-A study of an extremely pure preparation of Ricin. Jour. exp. Med. 1910,12,p.551.

Flexner, S.-The pathologic changes caused by certain so-called toxalbumins.-Medical News,1894,65, p.116.

Flexner, S.-The histological changes produced by ricin and abrin intoxications.-Jour. exp. Med. 1897,2,p.197.

Flexner, S.-The pathology of toxalbumin intoxications.-Johns Hopkins Hospital Reports.1897, 6 p.259.

Ford, W.W.-Plant poisons and their antidotes.-Centrall.f. Bak., Parasit. & Inf. 1913, 58. I, Ref. p. 129.

Fornace, L.-Contributo clinico-sperimentale allo studio dell' avvelenamento da serni di ricino.-Clin. med. ital., 1906, 45, p. 405.

Foster, N.B.-Studies of the influence of various dietary conditions on physiological resistance.-Jour. Biolog. Chem., 1910, 7,p.379.

Fraenkel, A.-Ueber die Wirkung des Ricins auf Fischblut.-Beitr. z. chem. Physiol. u. Pathol. 1904, 4, p. 224.

Fröhner, E.-Lehrbuch d. Toxikol. f. Tierärzte.-3d ed. 1910, p. 298.

Fröhner, E.-Toxische Rehe durch Rizinussamen.-Monatsch. f. prakt. Tierheilk, 1914, 26, p. 13.

Führer, H.-Nachweis and Bestimmung von Giften auf Biolog. Wege. 1911.

Führer, H.-Handbuch d. biochem. Arbeitsmethoden. 1911, 5, pp.28, 117.

Gebb, H.-Active Immunisierung vom Konjunktivalsack aus mittles Toxinen.-Arch. f. Augenheilkunde 1909, 64, p. 310.

Girard - Mangin and Henri, V.-Agglutination des Globules rouges par la ricine.-Compt. rend.

soc. biol. 1904, 56, vol. 1, p. 974.

Gullan, A. G.- Acute poisoning by a single castor oil seed. British Med. Jour, 1905, i, p,988,1188.

Guyot, G.-Ueber die Agglutinahilität der mit Formalin fixierten rot Blutkörperchen und der Blutkörperchenstromkt.-Centrbll. f. Bakt., Abt., 1, Orig. 1908, 48, p. 330.

Hamlin, M. L.-Analysis of the ash of the castor bean.-Biochem. Bull. 1912-1913, 2, p. 410.

Hanschmidt. E.-Zur Wirkung der Lecithine dei Vergiftungen der höheren Tiere.-Biochem. Zeitsch. 1913, 51,p.171.

Hausmann, W. and Kolmer, W.-Ueber die Einwirkung Kolloidaler Gifte auf Parämacien.-Biochem. Zeitsch. 1907, 3,p. 503.

Houze de l'Aulnoit.-Empoisonnement letal par les graines de ricin.-Arch.gen. de Med. 1869, March, p. 284.

Hutchinson, L.T.R.-Poisoning by castor-oil seeds.- Brit. Med. Jour. 1900, 1, p. 1155.

Jacoby, M.-Ueber die chemische Natur des Ricins.-Arch. exp. Path. u. Pharmakol. 1901, 46, p. 28.

Jacoby, M.-Ueber Ricin-Immunität.-Beitr. z. chem. Physiol. u. Pathol. 1902, 1, p. 51.

Jacdby, M.-Ueber Ricin-Immunitat, zweite

Mitteilung.-Beitr. z. chem. Physiol. u. Pathol. 1902, 2, p. 535.

Jacoby, M.-Ueber die Empfindlichkeit und des Rezeptions-vermögen der Zellen bei normalen und immunissietten Tieren.-Beit. z. Chem. Physiol. u. Pathol. 1904, 6, p. 113.

Jacoby, M.-Ueber Phytotoxine.-Biochem. Centrbll. 1903, 1, p. 289.

Jacoby, M.-Ueber die Beziehungen der Cerdauunswirkung und der Labwirkung.-Biochem. zeitsch. 1906, 1, p. 53.

Jacoby, M.-Ueber den Nachweis des Trypsins Biochem. Zeitsch, 1908, 10, p. 229.

Jacoby, M.-(Kraus & Levaditi) Handb. d. Technik. u. Methodik. d. Immunitätsforschung 1908, 1, p. 311.

Jodlbauer, A.-Die sensibilisierende Wirkung fluoreszierender. Stoffe.-Jahrb. d. Fortschr. a.d. Gebiete der physikal. Med. 1903, 1, p. 280.

Jodlbauer, A. and Tappeimer, H.-Die Beteiligung d. Sauerstoffes bei der Wirkung fluoreszierender Stoffee.- Deut. Arch. f. kl. Med. 1905, 82, p. 520.

Jodlbauer, A. and Tappeimer.H.-Ueber die Wirkung fluoreszierender Stoffe auf Toxine.-Deut = Arch. f.kl. Med., 1905, 85, p. 339.

Kober, P.A.-Pervaporation, perstillation and perorystallization. Jour. Amer. Chem. Soc. 1917, 39, p. 944.

Kobert, R.-Lehrbuch der Intoxikationen, 2d. ed. 1906, v. 2, p. 695.

Kobert, R.-Ueber vegetabilische Blut-agglutinine.-Stzber. d. Naturdorsch. Gesell. zu. Rostock, 1900, No.3 (Anh. z. Arch. d. Verein d. Freunde d. Naturg. in Mecklenburg).

Kobert, R.-Ueber Haemocyanin nebst einigen Notizen über Haemerythrin. Arch. f. d = ges. Physiol. 1903, 98, p. 411.

Kobert, R.-Einige Notizen über den biologischen Nachweis von Vegetabilische Agglutininen und Hamolysinen.-Landw. Vers. Stat. 1909, 71, p.257.

Kobert, R.-Beitrage zur Kenntniss der vegetabilischen Haemagglutine. Landwirt, Versuchstat., 1913, 79-80, p. 97. (Reprinted, Berlin, 1913).

Kraus, R.-Zur Theorie der Agglutination.-Zeitsch. f. Heilkunde 1902, 23, p. 369.

Kumagai, T.-Weitere Beiträge zur Wirkung von Eiweissziften auf isolierte Organe.-Zeitsch.f. Immunitätsforsch. 1914, 22, p. 306.

Landsteiner, K.-Ueber Serumagglutinen.-Munch. med. Woch. 1902, N. 46

Landsteiner, K.-Beobacht. über Hämagglutinatinen.-Wein. kl. Rundsch. 1902, Nr. 40 .

Landsteiner, K.-Hämagglutination und Hämolyse.-Handbuch d. Biochemie ()ppenheimer) 1910, 2, p. 395.

Landsteiner, K. and Jagic, N.-Ueber Reaktionen anorg. Kolloide u. Immunkörperreaktionen.-Münch. med. Woch., 1904, Nr. 27.

Landsteiner,K. and Pauli, W.-Elektrische Wanderung der Immunstoffe.-Verhand. d. Kongrese f. inn. Med. 1908, 25, p. 571.

Landsteiner, K. and Raubitschek, H.-Beobactungen über Hämolyse and Härnagglutination.-Centrabl. f. Bakt. 1 Abt. orig., 45. p. 660.

Landsteiner, H. and Reich, M.-Ueber Verbindungen der Immunkörper.- Centrbl. f. Bakt. Abt. 1, Orig. 1905, 39, p. 83.

Landsteiner, K. and Stankovic, R.-Ueber die Adsorption von Eiweisskörpern und über Agglutininverbindungen.-Centrbll. f. Bakt. Abt. 1, Orig. 1906, 41,p. 108.

Landsteiner L. and Stankovic, R.-Ueber die Adsorption v. Eiweisskörpern etc.-Centrbll. f. Bakt. Orig. 1906, 41,p. 108.

Langenfeldt, O.-Ein Vergiftungsfall in

Folge Genusses der Samenkörner des Ricunusstrauches.- Berl. kl. Wochensch. 1882, 19,p. 9.

Lav. C.-Ueber vegetabilische Blutagglutinine. Dissert. Rostock, 1901

Launoy, L.-Action antitryptique de serum sanguin chez les lapins intoxiques par la ricine.- C.R. soc. biol. 1911, 70, p. 367.

Lawrow, D.M.- Zur Frage über die Beeinflussung der Wirkung von Medikamenten durch Lecithine.- Biochem. Zeitsch. 1913, 54, p. 16.

Levaditi, C.,and Mutermilch, St.-Action de la ricine sur la vie et la multiplication des cellules in vitro.-Compt. rend. soc. biol. 1913, 74,p.611.

v. Liebermann, L.-Sind Toxine Fermente?.- Deut. med. Wochensch. 1905, 31, p. 1301.

v. Liebermann, L.-Ueber Hämagglutination and Hamatolyse.-Biochem. Zeitsch. 1907, 4, p. 25.

v. Liebermann, L.-Ueber Haemagglutination.- und Haematolyse.-Arch.f. Hygiene. 1907, 62, p. 277.

v. Liebermann, L.-Haemagglutination and Haemolyse.-Centrbl. f. Bakt. Abt.1, Orig. 1908, 47, p. 372.

Loewenstein, E.-Ueber die Bedeutung der cellulären Immunität. Prager med. Wochensch. 1901, p. 375

Madsen, T. and Walbum, L.-De la ricine et

de l'antiricine.-Centrbll. f. Bakt. 1st Abt. Orig. 1904, 36,p . 241.

Marsden, P.H.-Poisoning by a single castor oil seed.-Brit.med. Jour.1905, 1, p. 1299.

Meldrum, W.P.-Poisoning by castor-oil seeds Brit. Med. Jour. 1900, i, p. 317; Indian Med. Rec. 1900, 18, p. 220.

Mendel, L.B.-Vegetable Agglutinins.-Jour. biolog. Chem. 1909, 6, p. XIX.

Mendel, L. B.- Observations on Vegetable Agglutinins. Arch. di. Fisiol. 1909, 7, p. 168.

Michaelis, L. and Oppenheimer, C.-Ueber Immunität gezen Eiweisskörper Arch. f. Physiol. 1902, Suppl. p. 336.

Michaelis, L. and Steindorff, K.-Ueber die Wirkung des Rizins auf Serum und Organzellen in Vitro.- Biochem. Zeitsch. 1907, 2, p. 43.

Meissner,-Ueber die Giftigkeit der Ricin-ussammen.-Mitteil, d. Kaiser Wilhelms-Institutes f. Landwirtschaft zu Bromberg. 1909, 1, p. 217.

Miessner, -Die Giftizkeit der Rizinus-samen.-Mitt. d. Kaiser Wilhelm-Inst. in Bromberg. 1911, 3, p. 260.

Miessner, and Rewald. -Die Konglutination der roten Blutkörperchen.- Zeitschr. f. Immunitätsforsch 1909, 2 , p. 323.

Mooser, W.-Der Nachweis giftigen Rizinushestandteile in Futtemitteln. Landwirt. Vers. Stat. 75, p. 107.

Müller, F.-Beiträge zur Tokikol, des Ricins. Arch. f. exp. Pathol. u. Pharmakol. 1899, 42, p. 302.

Müller, F.-Ueber einige pathologisch-anatomische Befunde bei der Ricinvergiftung.-Beitr. z. Path. Anat. u. allg. Pathol. 1900, 27, p. 331.

Müller, W.-Studien ueber die Giftigkeit der Rizinussamen und den Nachweis des Giftes.-Deut. tierärzt. Wochenschr. 1911, 19, pp. 433, 449, 463.

Nagel, O.-Jour. Soc. chem. Ind. 1902,21,p,30

Neuberg, C.-Lipolyse, Agglutination und Haemolyse, IV, -Biochem. Zeitsch. 1908, 11, p. 400.

Neuberg, C.,and Rosenberg, E.-Lipolyse, Agglutination and Haemolyse I.Berlin, kl. Woch. 1907, 44, p. 54.

Nicolle, M.,Cesari, E. Etudes sur la ricine. II. Intoxikation ricinique chez le coboye.- Ann. Inst. Pasteur, 1913, 27, p. 358.

Nicolle, M. and Truche, Ch.-Note sur la conservation des "toxines solubles".-Ann. Inst. Pasteur, 1910, 24, p. 921.

Nicolle, M. and Truche, Ch. Seconde Note sur la conservation des "toxines solubles".-Ann. Inst. Pasteur,

1912, 26, p. 1030.

Officer, .-Poisoning by caster oil seeds. Australas Med. Gaz. 1895, 14,p. 283.

Olmer, D. and Sauvan, A.-Action "in vitro" sur le sang des solutions d'abrine et de ricine chauffies. Compt. rend. soc. biol. 1910, 68, 638.

Olmer, D. and Sauvan, A.-Action "in vitro" sur le sang des solutions d'abrine et de ricine, en presence de lecithine.-Compt. rend. soc. biol. 1910, 68, p. 639.

Oppenheimer, C.-Toxine und Antitoxine.- 1904.(also Mitchell's translation, 1906).

Osborne, T.B. and Mendel, L.B.- Ricine.- Amer. Jour. Physiol. 1903, 10, p. xxxvi.

Osborne, T.B., Mendel, L.B., and Harris, I A study of the proteins of the caster bean, with especial reference to the isolation of ricin.- Amer. Jour. of Physiol. 1905, 14,p. 259.

Osborne, T.B.- Crystallized vegetable proteids.-Amer. Chem. Jour. 1892, 14, p. 662.

Osborne, T.B.- The Vegetable Proteins, London, 1909.

Osborne, T.B.-Die Pflanzenproteine.- Ergebnisse der Physiologie, 1910, 10, p. 47.

Osborne, T.B.-Handbuch der bioch. Arbeits-

methoden, 1910, 2, p. 331.

Pascucci, O.-Ueber die Wirkung des Ricins auf Lecithin.-Beitr.. chem. Physiol. u. Pathol. 1905, 7, p. 457.

Pecholier, .-Etude sur l'empoisonnement pas les semences de ricin.-Montpelier med. 1869, Dec. p. 508.

Raubitschek, H.-Zur Kenntnis der Immumanti-phytalbumine.- Wien. klin. Wochensch. 1909, p. 1065.

Raubitschek, H.-Die Hamagglutination.-Zeitsch. f. Immunitätsforsch., Ref. 1910, p. 181.

Raubitschek, H.-Studien über Haemagglutination.-Zeitsch. f. Immunitätsforsch. Orig. 1911,9,p,297.

Raubitschek, H. and Russ, V.K.-Ueber entgiftende Eigenschaften der Seife.-Zeitsch. f. Immunitätsforsch. 1909, 1, p. 395.

Raubitschek. H. and Wilenko, M.-Zur Kenntniss der haptophoren Gruppen der agglutinablen Substanz.-Zeitsch. Immunitätsforsch. Orig. 1911,11,p,375.

Regenbogen, .-Berl. tierärzt.Wochensch. 1888.

Rehns,J.-L'absorption des toxins, agglutinines etc., injectees auniveau des voies respiratoires.-Compt. rend. soc. biol., 1901, 53, p. 687.

Rehns, J.-Contribution a l'etude des toxal-

bumines vegetales (abrine and ricine)-Compt - rend. soc. biol. 1902, 54, p. 212

Reid, G.-Beiträge zur Kenntniss der chemischen Natur und des biologischen Verhaltens des Ricins Landw.-Ver. Stat., 1913, 82, p. 393.

Ritthausen, H.-Die Eiweisskörper der Getreidearten, Hülsenfrüchte und Oelsamen, 1872.

Ritthausen, H.-Ueber die Eiweisskörper der Ricinussamen der Proteinkörper, sowie der Krystalloide dieser Samen.-Arch. f.d. ges. Physiol. 1879, 19, p. 15.

Ritthausen, H.-Krystallinische Eiweisskörper aus verschiedenen Oelsamen. Jour. f. Prakt. Chemie 1881, 23, p. 481.

Rochat, G.Fr.-Bijdrage tot de kennis van het werksame bestanddeel der ricine.-Nederl. Tydschr.v. Genesk. 1902, II, p.215. Also Qnaug. Diss. Utrecht 1902 (Maly's Jahresber. 1902, 32, p.942).

Roemer, P.-Experimentelle Untersuchungen über Abrin (Jequiritol) - Immunität als Grundlagen einer rationellen Jequirity - Therapie. Graefe's Arch. f. Ophthalmologie, 1901, 52, p. 72.

Sachs, H.-Die Haemolysine und ihre Bedeutung für die Immunitätslehre.-Lubarsch -Ostertags Ergebbissen der path. Anat. 1902, 7.

Stassano, H.-Sur les combinaisons des

nucleines avec les composes metalliques, les alcaloides et les toxines.-C.R. Acad. Sci. 1900, 131, p. 72.

Stepanoff, A.-Etudes sur la ricine et l'antiricine.-Ann. Inst.-Pasteur, 1896, 10, p. 663.

Stepanoff, A. L.- Ueber die Wirkung des Ricins bei Vergiftung und Immunization gegen Leukocytose Diss.)Russian) St. Petersburg, 1899; Wratsch, 1900 No.4) Merck's Jahresberischt, 1900)

Stillmark, H.-Ueber Ricin.-Arbeiten d. Pharmakol. Institut zu Dorpat, 1889, 3,p. 59

v. Tappeimer, H.-Ueber die Wirkung fluoreszierender Subst. auf. Fermente u. Toxine.-Ber. d.deut. Chem. Fesellsch 1903, 36, p. 3035.

v. Tappeimer, H. and Jodlbauer, A.- Die sensibilisierande Wirkung fluoreszierender Substanzen 1907.

Taylor, A.S.-Principles and Practice of Medical Jurisprudence, 2nd edition 1873, v.1.p. 328.

Tedeschi.-Avvelenamento sperimentale con la ricine.-Arch. di Farmacol. 1897, 5, p. 40.

Tichomiroff, M.-Ueber die Fallung von Toxalbuminen durch Nucleinsaure.-Zeitschr. f. physiol. Chemie, 1895, 21, p. 90.

Truche, Ch.-Etudes sur la ricine I. Preparation de serums antitoxiques, leur activite. Ann. Inst.

Pasteur, 1913, 27,p. 226.

Truche, Ch. and Alilaire, E.-Immunite hereditaire de la chevre vis a vis de la ricine.- Annal. Institut Pasteur, 1911, 25, p. 190.

Valenti. .Sulla azione di alcune sosterze tossische sulla conjunctiva oculaire.-Archivio di othalmol. 900, 8.

Vines, S.H.- On the proteid substances contained in the seeds of plants.-Jour. of Physiol. 1881 3,p. 93, Proc. Roy. Soc. London, 1879-80,30,p. 387.

Vitrac. Empoisonnement per des semences de ricin.- Gaz. hebd. d. sc. med. de Bordeaux. 1903, 24, p. 585.

Watson, G.A.- Note on Ricin and Abrin expeiments.- Brit. Med. Jour. 1905, ii, p. 1091.

deWaele, H.-Recherches sur l'anaphylaxis contre les toxines et sur le mode d'adsorption des toxines Zeitsch, f. Immunitätsforch. Orig. 1909, 3, p. 478.

Werner, E.-Ueber das Ricinin und den wirksamen Bestandteil der Ricinussamen.-Rharmaz. Zeitsch. f. Russland, 1870, 9, p.33.

White, J.S.-Bacterial, vs. vegetable toxines Pharm. Jour. 1915, 94, p. 156.

Weinhaus, O.-Zur Biochemie des Phasins.- Biochem. Zeitsch. 1909, 18, p. 228.

Wilenko, M.-Ueber das Präzipitationsvermögen pflanzlicher Eiweiss-stoffe.-Zeitsch f. Immunitätsforsch Orig. 1910, 5, p. 91.

Wolfson, E.M. (cases of poisoning by Ricinus) Wratsch, 1891, 12, p. 1037.

Wood, W.A.- Ten cases of poisoning by castor oil seed.- Australian Med. Jour. 1911, 16, p. 176.

Woronzow, W.-Zur Frage uber die chemische Natur des Ricins. Sitzber, d. Naturforscher Ges. d. Univ. Jurjew, 1908, p. 49, (Maly's Jahresber.,1908, 38, p. 1023.